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PROTOPLASM

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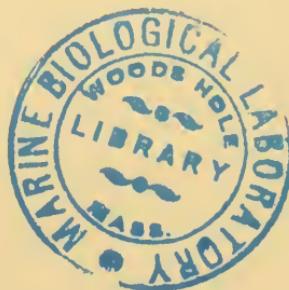
By

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who have shown so sympathetic and
helpful an interest in its preparation.*

PREFACE

"If, therefore, anyone wishes to search out the truth of things in serious earnest, he ought not to select one special science; for all the sciences are conjoined with each other and interdependent."

Thus wrote the French philosopher René Descartes. The purpose of this book is to show the truth of this statement in the study of protoplasm. On the pages that follow are brought together all those parts of the branches of science which bear upon the physical chemistry of living matter. Obviously, there is a space limit to what can be told, but at least enough will be said to indicate how far research has gone in the application of physics and chemistry to those biological phenomena which can be reduced to cellular or protoplasmic processes.

The presentation is as nontechnical as is consistent with accuracy and completeness. Mathematical formulas, curves, and tables have not been omitted—it would be difficult to do so in a number of instances where the subject defies a wholly nontechnical presentation—but they have been resorted to as infrequently as possible. A mathematical formula or a graph may, at first sight, be somewhat awe-inspiring, yet it often presents a complex situation in a simple and intelligible manner. On the other hand, it is equally true that a situation which can be clearly put into words becomes meaningless to the non-mathematical mind when stated in terms of formulas and curves.

The readers for whom this book has been written are students in biology and medicine and the related fields of biophysics and biochemistry. While it is not intended for my colleagues, should this volume fall into their hands, I trust they will find it of interest.

A list of references is appended, in case the reader should care to delve more deeply into the subject of this or that chapter.

Every effort has been made to present both sides of a question, yet with emphasis on that side which is favored by the author, in the belief that a definite but not too arbitrary stand is more conducive to the clear grasp of a subject than a wholly impartial point of view. With opposing evidence presented, the reader can judge for himself. A book wholly devoid of contention is likely to be less stimulating than one which occasionally indulges in healthy disagreement. A sincere attempt has, however,

been made to avoid severely adverse or destructive criticism and finality in statement, for both are out of place in science, especially today, when change and doubt are the very spirit of scientific thought, when the "immutable" elements are known to be mutable, when constancy in size and weight is no longer a necessary quality of the molecule, and when the laws of thermodynamics are being questioned. Laws and definitions should be clear and precise but not final; otherwise we fall into the dogma which dominated the science of the last century.

Needless to say, a volume such as the present one is the work of one man only in so far as he has penned it. The information given on the following pages is the result of the work of a large number of investigators. Many of them are mentioned, but many more are omitted. To name only the outstanding workers in the numerous and diverse fields of endeavor discussed in this book would be a futile task and would defeat the purpose of the book. An encyclopedic account of the subject is not our present purpose. The author has mentioned those investigators whose work he knows best, fully realizing that another author would make a different selection. After all, names add but a human touch, always delightful in any historical account but never important in the advancement of knowledge.

Writing about the experiments of others is a very pleasant task when one has the assistance of the workers themselves. The richest possession of a scientist is the sympathetic interest of his colleagues. Such an interest has made this book possible. Were full acknowledgment given to all for help received, the mere list of names would defeat its own purpose. Several of my coworkers have, however, given such substantial assistance that I wish to single them out for an expression of gratitude, without being unmindful of the many others to whom I owe much. I am particularly grateful to Professor Herbert Freundlich, who has read a major part of the manuscript of this book and contributed much to accuracy in statement, especially in those chapters dealing with strictly colloidal phenomena. To Professor Howard Pulling and Dr. Laurence Moyer I am greatly indebted for the reading and criticizing of a number of chapters.

PHILADELPHIA, PA.,
December, 1935.

WILLIAM SEIFRIZ.



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PROTOPLASM

CHAPTER I

THE LIVING SUBSTANCE

Protoplasm is living matter in its simplest form. When it occurs, as it nearly always does, in the form of an organized droplet, it is known as a *cell*, or *protoplast*. Cells, when permanently joined together, constitute *tissues*, and tissues unite to form *organisms*. Some cells, such as bacteria and amoebae, are complete organisms in themselves. They are known as *unicellular* organisms. The living substance of which cells are composed is protoplasm. A too precise definition of protoplasm is likely to lead to confusion. Even the specialist finds himself embarrassed by a limited definition. Briefly put, protoplasm is the basic material of life. The word means "primitive form."

The French zoologist Dujardin is credited with having discovered protoplasm in the year 1835. He called it "sarcode." Undoubtedly, earlier investigators—the inimitable observer and portrait painter Rösel von Rosenhof, who drew Amoeba in 1755; O. F. Müller, who described living Amoeba in 1773; and Ehrenberg, pioneer protozoologist—saw the living substance, for that is all there is to an amoeba, but to Dujardin apparently belongs the credit of realizing that the material that he saw is the substance which gives life to a cell. Today, nearly a century later, Dujardin's description of protoplasm is as accurate as any that can be given. He said, "Je propose de nommer ainsi ce que d'autres observateurs ont appelé une gelée vivante, cette substance glutineuse, diaphane, insoluble dans l'eau, se contractant en masses globuleuses, s'attachant aux aiguilles de dissection, et se laissant étirer comme du mucus, enfin se trouvant dans tous les animaux inférieurs interposée aux autres éléments de structure."

Four years later, in 1839, the Bohemian physiologist Purkinje first used the word "protoplasm" to describe the living substance composing animal eggs and embryos. The German botanist von Mohl applied the word to the living substance in plant cells and showed that the "sarcode" of animal cells and the protoplasm of plant cells are essentially the same. Von Mohl characterized protoplasm as "niemals einen klaren wässerigen Zellsaft . . . sondern . . . eine zähflüssige . . . Masse." While opinions differ somewhat, it is still held by the great majority of

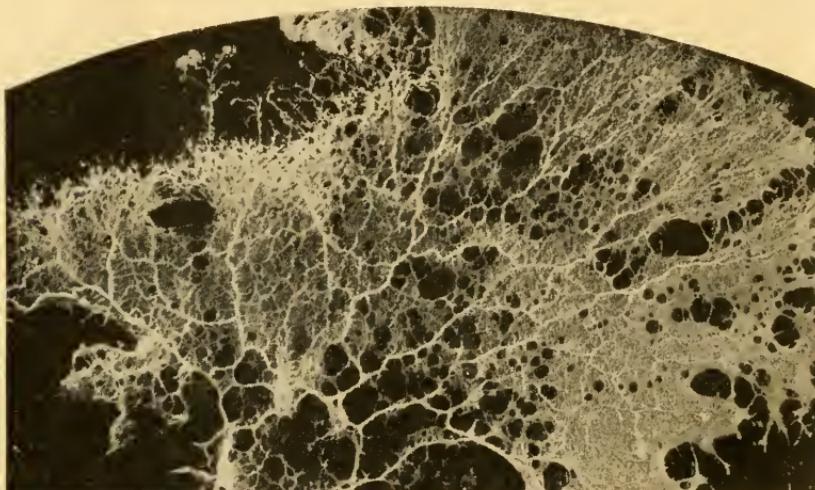


FIG. 1.—The naked protoplasm, or plasmodium, of a myxomycete (slime mold).

workers that protoplasm is what Dujardin and von Mohl said it was—a jelly, usually soft or fluid rather than firm though often tough and highly elastic, glutinous and fibrous, and taking up water with avidity.

The most extraordinarily intrinsic quality of protoplasm is its apparent and, in part, actual uniformity wherever found. Whether it is the protoplasm of the petal of a flower or of the muscle of an animal, it looks very much the same. Profound differences must, of course, exist; otherwise, why should one cell grow into a frog, while another very much like it in superficial appearance grows into a tree? The most fundamental differences in protoplasm are wholly hidden from us, but other differences less significant, yet often very characteristic, are evident. These latter have to do chiefly with such properties as con-

sistency, type and abundance of inclusions, and reactions to chemicals. Facts gained from a knowledge of these and similar properties give us all the insight we have into the structure of protoplasm and the mechanism of protoplasmic behavior.

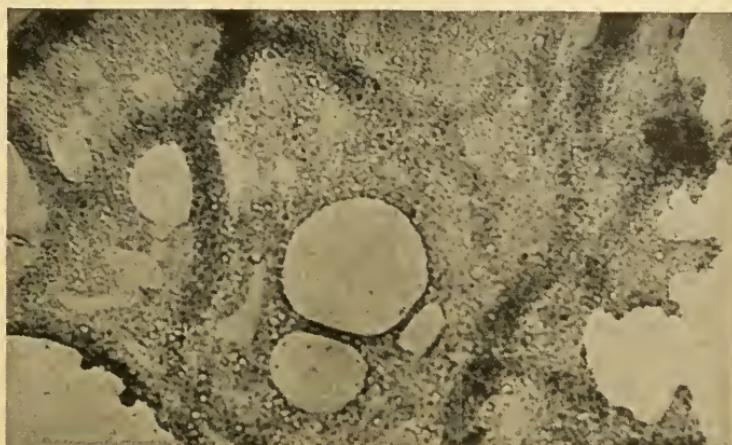


FIG. 2.—A small portion of the protoplasm of a myxomycete (at higher magnification than in Fig. 1). The largest oil globules (of 10μ diameter) appear as bright spots. (The two or three large circular areas are openings in the plasmodium.) The channels of flow are broad bands of darker color. (From J. Comandon.)

Protoplasm (Fig. 1) is a translucent, grayish, and slimy substance, usually capable of flowing freely, though often of moderately high consistency even when flowing. It looks much like the white of an egg though less homogeneous, as only rarely is it devoid of visible inclusions. Under low magnification, protoplasm appears to be a dispersion of many globules and tiny granules in a thick matrix. The granules are often mere specks when seen under the highest magnification of the microscope. It is impossible always to say whether protoplasmic particles are actually granules or droplets (*i.e.*, whether of solid or liquid material). The distribution of granules and globules is usually a heterogeneous one, as in the plasmodium of a myxomycete (slime mold) (Fig. 2), but it may assume a certain degree of symmetry, as in echinoderm (*e.g.*, starfish) eggs (Fig. 3). Great care must be

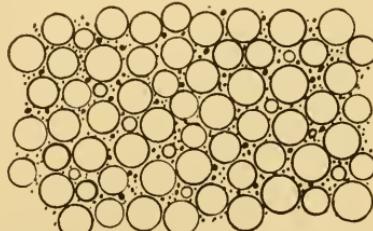


FIG. 3.—Sketch of the protoplasm of an Echinoderm egg.

taken in interpreting the superficial structure of protoplasm as revealed by the microscope, for the living emulsion presents a variety of forms which look so different from one another that several quite distinct types of structure have been ascribed to protoplasm, when, actually, most of them are different forms of the same thing (page 241).

Lying beyond the visible structure of protoplasm is another of a quite different character to which many of the more fundamental properties of living matter owe their existence. Of the nature of this "ultimate" structure we can only conjecture, though our speculations have some foundation. The subject of protoplasmic structure is of such importance that a separate chapter is devoted to it.

If a superficial examination is made of a protoplasmic mass, say an amoeba or a myxomycete, it will be noticed that there is an inner region of granular matter, which is called *endoplasm*, and a very narrow border of clear hyaline substance quite free of granules, which is termed *ectoplasm*. The latter appears to be simply the matrix of the former, which for some reason is kept free of particles at the periphery. Closer examination may reveal a delicate surface layer, the *protoplasmic membrane*, which dissection shows to be of greater consistency than the inner protoplasm. Plant cells, unlike animal cells, are usually surrounded by a heavy wall of cellulose (Fig. 4). In some unicellular organisms (Fig. 28), the outermost membrane assumes the proportions of a tough pellicle, roughly comparable to the cell wall of plants. In plant cells, and possibly also in Protozoa, the protoplasmic membrane lies just beneath the wall or pellicle.

Biologists are ever trying to distinguish finer differentiations in the structure of protoplasm. The German botanist Strasburger recognized two components, one of which he called *kinoplasm*, or "active" plasm, and the other *trophoplasm*, the "nurse," or nutritive, plasm. Searth and Lloyd have brought Strasburger's terms into use again and emphasized their significance. They defined the kinoplasm as the active irritable component and the trophoplasm as the nutritive substance. A similar differentiation between the smallest visible component parts of protoplasm has given rise to the names *phaneroplasm*, or visible plasm, and *cryptoplasm*, or hidden plasm, the former being the dispersed phase (liquid globules) and the latter the dispersion medium

(matrix) of a very fine emulsion (Fig. 121). Nearly all cells or organized droplets of protoplasm possess certain parts which are typical of them. Chief among these is the *nucleus* (Fig. 4). It is (usually) a large, central body of vital importance to the cell.

When speaking of protoplasm it is customary to include the nucleus and to use the word *cytoplasm* when the nucleus is to be excluded, though actually few biologists bother always to make the distinction. What has been said of protoplasm in the preceding paragraphs refers especially to the cytoplasm, though the properties of the nucleus are similar. It, too, is of a slimy, elastic, sometimes quite thin, sometimes thick material which is physically much like cytoplasm but differs from it in chemical constitution. It exists as a distinct globule within the cytoplasm, influenced by the latter just as it in turn influences, if it does not to some extent control, the cytoplasm. The nucleus is separated from the cytoplasm by a distinct nuclear membrane, which breaks down during cell division when there results a more intimate contact between cytoplasm and nuclear matter. During the time that the two substances are thus intimately associated, there is probably a marked influence of the one on the other. Possibly at this time cytoplasmic characteristics are transferred to the nucleoplasm and thus transmitted as hereditary traits.

Protoplasm usually appears to be quiet except for occasional minor disturbances, but there are times when it is exceedingly active, its activity taking the form of rapid flowing. Streaming protoplasm is a sight that never fails to hold the interest of a "protoplasmologist." The flow may be circular, as it is within a typical cell, or in only one direction at a time. The latter one-way streaming is usually characteristic of cells that are exceptional in form, such as the plasmodium of a slime mold (Fig. 1) or the long tubes of bread mold and certain algae (*Vau-cheria*). Protoplasmic streaming is very typical of some cells and is nearly always present in them (Amoeba and slime molds). In other cells, streaming is seldom if ever to be seen, though some movement of the protoplasm is likely to occur within every cell from time to time.

Protoplasm often develops superficial processes or growths which become at times very striking. These surface processes are formed, sometimes regularly and normally, when they may serve a useful purpose, and sometimes exceptionally and appar-

ently abnormally. The most conspicuous and well known among the former are the *pseudopodia*, or lobelike protrusions put out from the surface of Amoeba (Fig. 24) and like organisms by means of which they move. Similar but much finer are the protoplasmic protrusions from bacteria which may also serve for the purpose of locomotion. Other structures of the same kind have been quite extensively studied under the name of *filose threads* (of the Foraminifera and Radiolaria). The fact that certain fatlike substances (lipoids) form identical processes (myelin threads) (Fig. 176) has led to the belief that the surface of protoplasm is of such a material. Very interesting are the protoplasmic protrusions given off by unripe eggs of Echinoderms when in the presence of sperm (Fig. 175). Sperm are necessary to arouse the surface protoplasm into such activity. The structures are without evident function. Similar processes formed by the ripe egg awaiting fertilization may serve a purpose, to guide the sperm. All such activities indicate that the surface of protoplasm can be as active as is the inner protoplasm.

Studies on protoplasm may be chemical or physical. The physical properties of protoplasm are the subject of most of the chapters which follow. Certain properties have been ascertained which are as yet of purely theoretical interest; such are specific gravity determinations. Pfeiffer obtained values of these varying from 1.025 to 1.06, with 1.04 as an average.

Chemically, protoplasm is three-fourths water. Of the remaining solid matter, the greater part is protein, the rest being mostly carbohydrates (sugars), fats, and salts. The maximum water content of protoplasm cannot be stated with certainty; where it appears to be very great, as in the jellyfish, one cannot say how much of the organism is really protoplasm and how much just nonliving jelly. Gortner states that the jellyfish is a living system in which water plays a role probably more important than that of any other single constituent. The maximum water content of the jellyfish is given as 99.8 per cent; therefore, only 0.2 per cent is dry matter. The water content of tadpoles may be nearly as high. In man, the amount of total water is about 65 per cent, lowered to 53 per cent by old age or disease. A probable minimum water content of protoplasm is 8 per cent in dormant seeds. These may live for more than a hundred years. (Two hundred years for seeds of *Nelumbo*, the Japanese water

lily, is one of the few authentic records of old seeds still capable of germination.) The average seed contains from 15 to 30 per cent of water. An extraordinary example of minimum water content in protoplasm is that of the so-called *sclerotium* of slime molds. A slime mold may do one of two things when winter comes or when food is exhausted or unusual dryness prevails. It may form spores (reproductive cells), or it may dry out to a great extent and cease all visible activity. This dried protoplasm is known as the sclerotium, and it resembles nothing more closely than a bit of thin leather. It is exceedingly stiff and often breaks with the brittleness of glass. This fact gives one quite a different concept of living protoplasm from that commonly held. In this condition it may last for years, yet when given moisture, warmth, and food, it will start flowing and again become an active living sheet of protoplasm.

Among the earlier determinations of the chemical constitution of protoplasm is that of Reinke on the plasmodia of myxomycetes. This material was chosen because it is the only pure protoplasm which can be had in relatively large quantities. Animal bodies have much accessory material such as fat and sinew. The analysis of Reinke yielded:

	Per Cent		Per Cent
Proteins.....	55	Cholesterin.....	2
Fats.....	12	Resins.....	1
Carbohydrates.....	12	Salts.....	7
		Undetermined.....	11

The more recent work of Kiesel shows the composition of the dry (water-free) plasmodium of the slime mold *Reticularia* to be:

	Per Cent		Per Cent
Albumin.....	20.65	Lecithin.....	4.67
Plastin (protein).....	8.42	Cholesterin.....	0.58
Nucleic acids.....	3.68	Carbohydrates.....	8.06
Nitrogen-containing extracts	12.00	Glycogen.....	15.24
Oils.....	17.85	Unknown.....	8.85

The composition of the human body is 65 per cent water, 15 per cent protein, 14 per cent fat, 5 per cent salt, and 1 per cent unidentified matter, representing the following elements (in order of abundance): oxygen, carbon, hydrogen, nitrogen, calcium,

phosphorus, potassium, sodium, chlorine, sulphur, magnesium, iron, iodine, fluorine, silicon, manganese, arsenic, etc.

There is a tendency to regard protoplasm as a very simple physical system. To be sure, it is, as a whole, primarily a physical mixture and not a compound in the true chemical sense. In some respects, it resembles as simple a system as a solution of salt or sugar in water, but it is inconceivable that living matter is no more intricate than this. There is no direct optical evidence of great complexity in protoplasm, but if we view the question dynamically, and consider the extreme diversity in the form and behavior of organisms, reflecting for a moment on the manifold activities of a single cell, such as that in the brain of a man or in the leaf of a tree, it would seem that protoplasm must be a highly complex and superbly organized system.

Complexity in structure leads to the question whether or not this structure or any other property of protoplasm is characteristic of it alone, *i.e.*, of life. It is recognized that living matter is in certain respects quite distinct from nonliving matter. It is further recognized that a definite structural organization or specific constituent of protoplasm may be responsible for this distinction, but just what type of organization or particular constituent cannot be said. One aspect of this problem is seen in the attempts made to distinguish between what is alive and what is not alive in protoplasm and to restrict the term protoplasm so that it will include the living constituents only, but all such attempts are in the main futile. However, it does appear justifiable to regard certain of the constituents of protoplasm, such as a fat droplet, a starch grain, or a crystal, as lifeless. The water, which makes up three-fourths of protoplasm, is certainly not alive when considered apart as water, but some of it may be so intimately associated with other constituents of protoplasm as to become in a sense alive. Those protoplasmic centers where metabolic activity is greatest—centers of organic synthesis such as the chloroplasts where sugar is formed and the pyrenoids where starch is formed—would appear to merit the title of living as much as, if not more than any other part of the cell, but it is these very processes and the substances involved which are capable of the strictest physical-chemical interpretation. Chlorophyll may be viewed simply as a catalyst or merely as a color screen and thus robbed of all “vital” significance.

Attempts to differentiate between the living and the nonliving in protoplasm date from the earliest investigators. Hanstein distinguished between the active living protoplasm and the passive, lifeless "metaplasma." Sachs called the former "energid" and the latter "energid products." The term "metaplastic" (or "paraplastic") still persists for metabolic products which are generally recognized as lifeless. Other biologists speculating on this distinction have wandered far into the philosophical field by postulating special vital bodies which give to protoplasm the properties of life. Buffon and Verworn conceived of gigantic molecules termed "biogens" which were supposed to be the life-giving elements of protoplasm, the rest of the material being presumably nonliving. Spencer postulated "physiological units"; and Altmann, "bioblasts." In this category, though referring more particularly to hereditary units, belong the "gemmales" of Darwin, the "pangens" of deVries, the "plastidules" of Haeckel, the "biophores" of Weismann, and the "genes" of modern geneticists. Nägeli's "idioplasm" theory, while speculative like the others, has the advantage of resting on a chemical foundation which is in harmony with certain known facts. He accounted for hereditary traits by specific molecular orientation. This led further to a conception of elementary units of structure or aggregates of molecules, which Nägeli termed "micellae" and which were destined to play a prominent role in subsequent theories of protoplasmic and colloidal structure.

Older ideas on what in protoplasm is living and what not have been mostly discarded. The controversy has settled down to two possibilities, *viz.*, the presence of some one ultimate vital substance, on the one hand, or, on the other hand, a mixture of substances the individual components of which taken alone are nonliving but combined constitute a living substance. (There is always the third possibility that the constituents are nonliving, while the life force itself is extramundane). If the first possibility is true, then the vital substance is most probably protein in nature. Leathes states that proteins are generally considered the most important components of protoplasm. Pauli writes, "There can be no doubt as to the central position of the proteins in the organization of living matter. They alone display the specific properties of life." The opinion prevails among many



workers that a protein complex is the ultimate living substance. The constituents of this complex are probably of the nature of enzymes or organic catalysts.

The opposing viewpoint rests on the assumption that no distinction exists between the living and the nonliving in protoplasm, that the living substance is alive because it is an organized *system* the component parts of which are lifeless when considered individually but when associated in a coordinated state, life results. Hopkins says, "We can scarcely speak at all of living matter in the cell. At any rate we cannot . . . speak of the cell life as being associated with any particular type of molecule. Its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system. Life . . . is a property of the cell as a whole." One must, of course, agree with Hopkins' further remark that "the integrity of metabolic life of a liver cell is as much dependent on the existence of the metaplastic glycogen, however small in amount, as upon the existence of the nuclear material itself." So also is the running locomotive dependent upon its fuel of coal and water, but the ultimate mechanism is a structure of steel. Living protoplasm has its fuel and a mechanism for converting the fuel into potential energy. Wilson agrees with Hopkins in saying, "The term protoplasm does not designate a single substance but is a collective name for the sum total of the active components that cooperate in the work of a complex system; and life is the sum total of the activities of that system." Sharp believes it to be a fundamental fallacy to attribute the properties of a system to one or more of its constituent elements. Mast is equally firm in this belief. He states, "If, then, protoplasm is defined as living substance, its structure must involve the cell as a whole, not . . . this portion or that portion." So we conclude from this viewpoint that any substance acquires the property of life when it becomes part of a living system.

The opposing belief in a basic substance also carries with it the concept of protoplasm as a living *system*. If there is some one substance or a complex of substances which represents ultimate living matter, then it can maintain life only when it is surrounded by and intimately associated with its environment of water, fats, etc. One might compare protoplasm to a larger living system, such as a plant, where it is clear that the waxy cuticle of the leaves, the bark, the stored food, the resin, the latex,

and like matter are absolutely necessary for the well-being of the plant yet are not alive. Just so does protoplasm contain its nonliving constituents, its nutrient matter, its internal environment. Kiesel takes a very firm stand on the purely passive or independent nature of the free water in protoplasm, which he says is not a part of the "ground substance" of living matter. The water in vacuoles (about which there can be no doubt), water taken in by imbibition or osmosis, and perhaps also the water of hydration of colloidal particles Kiesel says is not a constitutional part of protoplasm but only a medium for its activities.

Vital staining, wherein dyes are added to the surrounding solution and taken up by cells, is presumed to give some indication of what is alive and what not. Metaplastic or nutritive substances, such as oil globules, are readily stained, while the protoplasmic matrix and the nucleus are not usually, if ever, stained when alive.

Associated with the problem of what in protoplasm is living and what not, is the problem, what organism is to be regarded as representing the simplest form of living individual. This honor is usually given by the zoologist to Amoeba. The botanist, however, regards the unicellular blue-green algae and the bacteria as the most primitive forms of life, with the slime molds (myxomycetes) not far above. The idea of an extremely elementary state of living matter more primitive than the forms just mentioned has long occupied the thoughts of biologists. Haeckel, "the German Darwin," found in the sea what he thought to be the most primitive form of protoplasm. He, a monist (monism is the doctrine of the identity of matter and mind), was quite ready for such a primordial mass of protoplasm, because it fitted in with his philosophical idea that life started in an undifferentiated bit of living matter. Here, actually now on earth, was, so he thought, his *Urschleim*. The supposedly primitive protoplasmic mass that Haeckel found in the sea was apparently devoid of a nucleus or other differentiation. He classed it as "Monera." Haeckel's Monera (*Bathybius haeckelii*) was either a marine Amoeba or an artifact (an artificial product), perhaps the slimy precipitate of a calcium salt. Though his find was not what he thought it to be, yet Haeckel's philosophical idea is nevertheless sound, for we cannot escape the conviction that life began in a relatively undifferentiated mass of "protoplasm."

CHAPTER II

THE CELL

Living things, or, as we say, *organisms*, are built of miniature units known as *cells*, just as a house is built of stones. Many very lowly organisms, such as bacteria, consist of but a single cell. All cells are delimited by a wall or membrane. The living matter within them is protoplasm. A cell may be defined as a

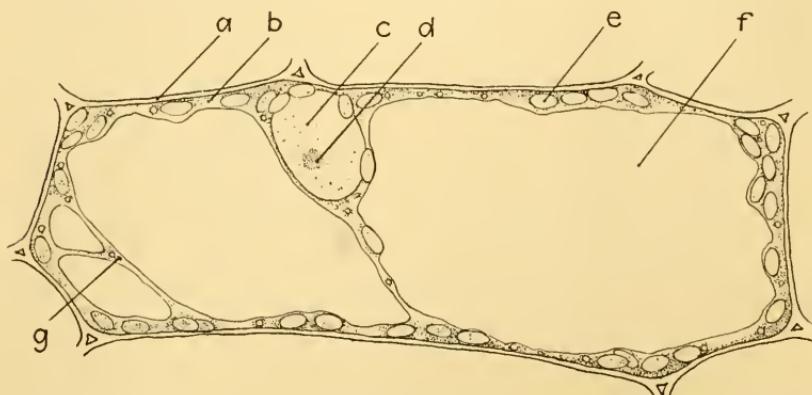


FIG. 4.—A typical plant cell (from the leaf of the water plant *Elodea*). *a*, The wall of cellulose, the inner surface of which is lined by the protoplasmic membrane; *b*, the protoplasm, termed cytoplasm when considered apart from the nucleus; *c*, the nucleus; *d*, the nucleolus; *e*, a (green) chloroplast; *f*, the large, central cavity or vacuole filled with an aqueous solution and traversed by protoplasmic strands, *g*. (Drawn by J. Plowé.)

protoplasmic mass enclosed by a membrane. Cells are usually of microscopic size, especially when they are parts of tissue. Only rarely, when existing as individual organisms, can they be seen with the unaided eye, and then usually simply as a mere speck. The typical plant cell possesses two coverings, a delicate, living protoplasmic membrane, and a heavy wall of nonliving cellulose (Fig. 4). Animal cells are usually enclosed within a delicate membrane only and have no outer heavy wall (Fig. 5). It was, by chance, the empty and dead cellulose housing of plant cells which was first seen and called a cell. In the year 1665, the

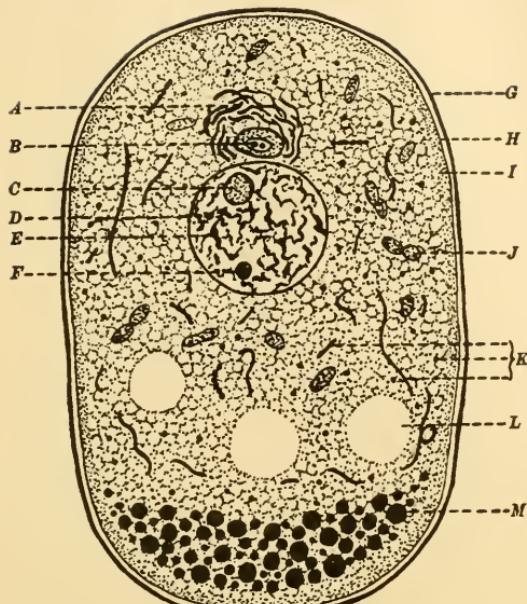


FIG. 5.—A schematized animal cell. *A*, Golgi body; *B*, centrosomes; *C*, true nucleolus (or plasmosome); *D*, nucleus; *E*, chromatin; *F*, chromatin nucleolus (or karyosome); *G*, cell wall (or pellicle); *H*, plasma membrane; *I*, cortical layer; *J*, plastids; *K*, chondriosomes; *L*, vacuole; *M*, passive (metaplastic or paraplastic) bodies (fat globules, etc.). (*From E. B. Wilson.*)

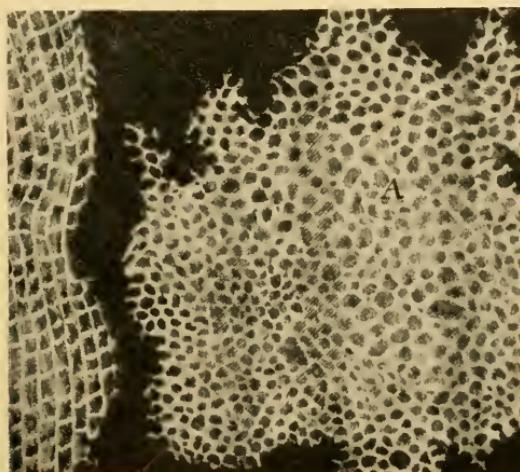


FIG. 6.—Part of Robert Hooke's first drawing of cells (in cork).

MICROGRAPHIA.

II3

Observ. XVIII. Of the Schematisme or Texture of Cork, and
of the Cells and Pores of some other such frothy Bodies.

I Took a good clear piece of Cork, and with a Pen-knife sharpen'd as keen as a Razor, I cut a piece of it off, and thereby left the surface of it exceeding smooth, then examining it very diligently with a Microscope, me thought I could perceive it to appear a little porous; but I could not so plainly distinguish them, as to be sure that they were pores, much less what Figure they were of: But judging from the lightness and yielding quality of the Cork, that certainly the texture could not be so curions, but that possibly, if I could use some further diligence, I might find it to be discernable with a Microscope, I with the same sharp Pen-knife, cut off from the former smooth surface an exceeding thin piece of it, and placing it on a black object Plate, because it was it self a white body, and casting the light on it with a deep *plano-convex Glass*, I could exceeding plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular; yet it was not unlike a Honey-comb in these particulars.

First, in that it had a very little solid substance, in comparison of the empty cavity that was contain'd between, as does more manifestly appear by the Figure A and B of the XI. Scheme, for the *Interstitia*, or walls (as I may so call them) or partitions of those pores were neer as thin in proportion to their pores, as those thin films of Wax in a Honey-comb (which enclose and constitute the *sexangular cells*) are to theirs.

Next, in that these pores, or cells, were not very deep, but consisted of a great many little Boxes, separated out of one continued long pore, by certain *Digibrages*, as is visible by the Figure B, which represents a sight of those pores split the long-ways.

I no sooner discern'd these (which were indeed the first *microscopical* pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this) but me thought I had with the discovery of them, presently hinted to me the true and intelligible reason of all the *Phenomena* of Cork; As,
First, if I enquir'd why it was so exceeding light a body? my Microscope could presently inform me that here was the same reason evident that there is found for the lightness of froth, an empty Honey-comb, Wool, a Spunge, a Pumice-stone, or the like; namely, a very small quantity of a solid body, extended into exceeding large dimensions.

Next, it seem'd nothing more difficult to give an intelligible reason, why Cork is a body so very unapt to suck and drink in Water, and consequently preserves it self, floating on the top of Water, though left on it never so long; and why it is able to stop and hold air in a Bottle, though it be there very much condens'd and consequently preses very strongly to get a paslage out, without suffering the least bubble to pass through its substance. For, as to the first, since our Microscope informs us that the substance of Cork is altogether fill'd with Air, and that that Air is perfectly enclosed in little Boxes or Cells distinct from one another. It seems very plain, why neither the Water, nor any other Air can easily infinuate it self into them, since there is already within them an *intus existens*, and consequently, why the pieces of Cork become so good floats for Nets, and stopples for Viols, or other close Vessels.

And thirdly, if we enquire why Cork has such a springiness and swelling nature when compris'd? and how it comes to suffer so great a compression, or seeming penetration of dimensions, so as to be made a substance as hevie again and more, bulk for bulk, as it was before compression, and yet suffer'd to return, is found to extend it self again into the same space? Our Microscope will easily inform us, that the whole mass

R

consists

FIG. 7.—Parts of pages from Robert Hooke's "Micrographia" (1665).

Englishman, Robert Hooke, while examining objects in general with his new and primitive microscope, chanced upon a piece of cork. He saw a myriad of tiny cavities, empty, with heavy walls (Fig. 6). He described them as "little boxes or cells distinct from one another" (Fig. 7). Thus were discovered the dead and empty chambers which are the skeleton of the plant. The living matter within them was not seen and understood until nearly two centuries later.

The study of cells constitutes that branch of science known as *cytology*.

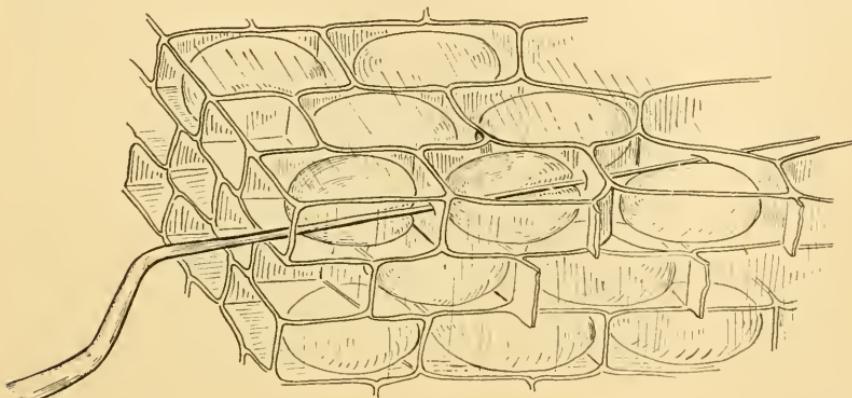


FIG. 8.—Drawing of a group of plant cells from an onion with the contents plasmolyzed (a fine glass needle is shown penetrating the walls of cellulose).

The Shapes of Cells.—Plant cells are usually angular in shape, their form being maintained by the heavy cellulose wall. The absence of such a wall permits animal cells to assume a globular form or at least a smooth contour. Plant cells are sometimes shaped like rectangular boxes (Fig. 8), but more commonly they are irregular 12- or 14-sided polyhedrons (Fig. 9). Work done on the shapes of plant cells by Frederick T. Lewis leads to the conclusion that the orthic tetrakaidecahedron, a 14-sided figure with eight hexagonal facets and six quadrilateral ones, is the most common form among plant cells. There are other unique shapes assumed by highly specialized cells, to be described later.

The Parts of Cells.—The hollow cells of seasoned lumber or cork constitute the nonliving cellulose skeleton of the plant. It is the contents of these skeletal cavities which give the property

of life to the plant. In order to distinguish more clearly between the dead cell of cellulose and the protoplasm which fills it, the term *protoplast* was coined to indicate the living contents of a cell considered as a unit. It is not used in the same sense as protoplasm, for the latter is the living substance in any form, while the protoplast is the complete, organized unit, that is to say, the living cell without its (heavy) wall.

That the living plant cell is really a protoplast—an individual organized droplet of protoplasm housed in a cellulose box—is easily proved by the process known as *plasmolysis*. If plant tissue is put in a salt or sugar solution which is of higher concen-

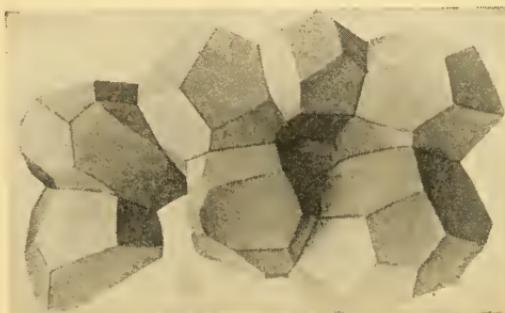


FIG. 9.—Drawing of a group of plant cells. (From F. T. Lewis.)

tration than the salt content of the cell (about 5 per cent potassium chloride or 18 per cent sugar), the cell loses water to the outside solution, and the protoplast shrinks away from the cell wall (Fig. 8). This takes place because the internal pressure, the *turgidity* of the cell, is reduced. The protoplast shrinks as does a toy balloon from which the air is slowly escaping. We learn from this experiment that the protoplast is an entity in itself and that the plant cell is not simply a cellulose container with fluid poured into it like water into a box. In addition to a wall of cellulose plant cells possess a protoplasmic membrane. Such a membrane exists at the surface of all protoplasts but is usually not distinguishable from the protoplasm within. In normal plant cells, the membrane is pressed tight against the inner surface of the cellulose wall. In plasmolyzed cells, the membrane pulls away from the wall with the protoplasm (Fig. 8). In most animal cells, the protoplasmic membrane is the outermost layer, or "wall," of the cell.

The typical cell possesses a nucleus (Figs. 4, 5, 10b). A few rare and unusual exceptions exist. The nucleus is ordinarily a spherical or ovoid body, centrally or peripherally located. It is an organ of fundamental importance to the continued life of the cell. If the nucleus is removed from the cell, as it can be under favorable conditions, the cell may continue to live but not for long, nor can it reproduce. It survives only as long as the nourishment lasts which it has taken in before the operation; this is about a week in the case of an amoeba. A typical cell has one and only one nucleus, but there are numerous exceptions of cells with several nuclei and some few types which have no nucleus. The red corpuscle of man is a non-nucleated cell. When the corpuscle starts life in the marrow of the bone it has a nucleus; but as it grows and migrates out into the blood stream the nucleus disappears. Whether or not the little sac of hemoglobin that remains is to be regarded as a living cell is as yet an unanswered question. The sieve tube in the wood of plants transports food; it is a living cell with active protoplasm, but when mature it has no nucleus. Certain cells possess two or more nuclei. The cells of the mushroom are binucleate, and the polymorphonuclear white blood cell has typically several nuclei. There are unusual cases of cells with as many as six or more nuclei (Fig. 10a).

The typical nucleus contains a tiny body called the *nucleolus*, or "little nucleus." There may be several nucleoli (Figs. 5, 10a, 10b). The functions of the nucleolus are unknown, though many have been attributed to it.

Another important cell structure, much more conspicuous and probably more common in plants than in animals, is the *vacuole*, poorly named, for a vacuum is impossible in the living world. The name roughly carries the idea of a cavity. The vacuole in most plant cells is a single, large, centrally located sac filled with salts, sugars, and organic substances in aqueous solutions (Fig. 4). It plays a very important part in the life of the cell. Owing to its significance, its widespread occurrence, and variations in form, we shall return to it for a more detailed discussion later.

Among the conspicuous plant-cell inclusions are the *chloroplasts* ("green bodies") which occur in all green tissues of plants, therefore most commonly in leaves (Fig. 4). These little globules contain *chlorophyll*, the substance primarily responsible for the

manufacture of food in plants. A fundamental distinction between plants and animals is the ability of the former to manufacture food (organic matter) from inorganic material. This is possible because of the presence in plants of chlorophyll which, acting as a catalyst, or stimulator of chemical reactions, converts water and carbon dioxide into sugar with the aid of the sun's energy. Chloroplasts do not occur in typical animal cells.

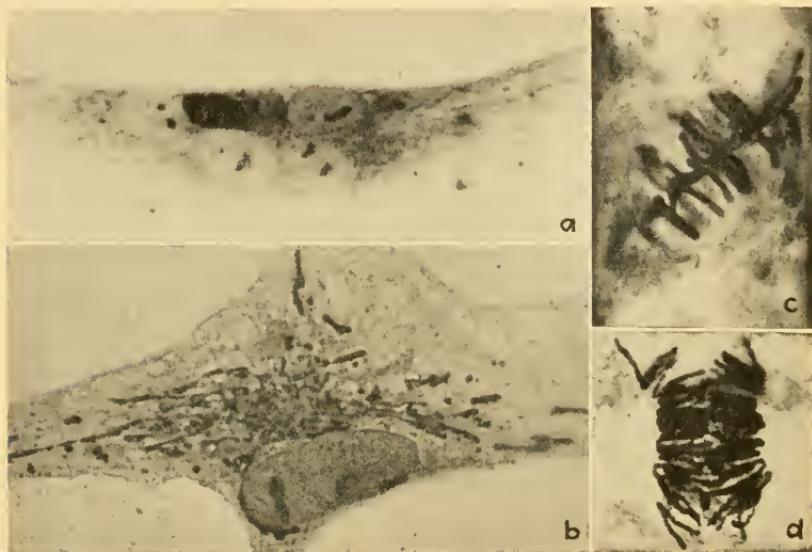


FIG. 10.—*a*, A cell (fibroblast in culture, boundary indistinct below) with six nuclei, all of which have two or more nucleoli; *b*, animal cell (fibroblast, a connective tissue cell from a chick embryo culture) with mitochondria: 1450 \times (from Warren Lewis); *c*, chromosomes of pine (cambium) beginning to migrate from the equatorial plate; *d*, chromosomes of pine (cambium) at the equatorial plate. (*c* and *d* from I. W. Bailey.)

The chloroplast is one type of *plastid*, or "small body," in cells. Other more minute ones are *mitochondria*, or *chondriosomes*, which have enjoyed considerable scientific publicity. They are granules, rods, or threads of varying size and shape occurring in practically all cells (Figs. 5, 10b). What they really are no one knows. Guilliermond has made a very extensive study of mitochondria and believes them to play an important part in the life of the cell. Being motile, they were once believed to be independent organisms, like a colony of bacteria enjoying the hospitality of a host, but this belief lacks convincing evidence. The names given to them are legion. At least half a hundred

appellations have been applied to mitochondria based on their size, shape, where found, supposed function, etc. Mitochondria have been thought to give rise to other plastids within the cell, such as the chloroplasts. They have also been credited with the important function of carrying hereditary traits, but the evidence for this is also far from conclusive. The most convincing hypothesis on the function of mitochondria is that which ascribes to them a purely metabolic activity in which they are concerned with digestion, functioning as starch-splitting enzymes (*e.g.*, in seed germination), or they may be, as Lloyd and Scarth believe, reserve supplies of lecithin for the protoplasm.

The sometimes rod-shaped, sometimes netlike structure called the *Golgi apparatus* (Fig. 5), discovered by an Italian of this name, bears often a superficial similarity to mitochondria. Its origin and function are not known; indeed, like some other cell parts, its existence as a normal cell inclusion has been doubted ever since its discovery, but anyone who has seen the Golgi apparatus in all its forms, in a variety of cells, such as those exhibited by de Fano, cannot but acknowledge that whatever the Golgi apparatus may be, it stands for a definite structure in the living cell. It appears to be of rather general occurrence in animal cells. While more typical of higher animals, it has been described as occurring in Protozoa (Endamoeba, the Amoeba found in the sublingual spaces of our mouth). Whether or not there is any counterpart of the Golgi apparatus in plant cells has been answered in the affirmative by Drew, who finds structures in plant cells (when certain preparation methods are used) which he believes to be analogous to the Golgi apparatus in animal cells. The extraordinary vacuoles of varied shape which I. W. Bailey has seen in plant cells (Fig. 18) so closely resemble some forms of the Golgi apparatus that there is some possibility of the latter also being vacuoles.

Other granules and globules occurring within cells are vesicles, fat droplets, yolk globules, pigment granules, starch grains, starch-producing centers (the pyrenoids in plants), crystals, etc. The crystals formed by plant cells may be very striking (Fig. 11).

There is a most significant cell process which reveals another exceedingly important cell structure. When cells divide they go through, in nearly all cases, a process known as mitosis during which there appear structures called *chromosomes*. They are

wormlike bodies (Fig. 12) which are clearly visible only during cell division. The nucleus of a "resting" cell (the term is a misnomer, as the so-called "resting" cell may be very active in other ways) is frequently devoid of evident structure (the resting animal nucleus is usually optically empty, but the plant nucleus most often reveals structure). When cells are about to divide and produce two daughter cells, a marked change takes place. The *chromatin* material (Fig. 12a) of the nucleus collects into a long thread called the *spireme* (Fig. 12b) which breaks up into

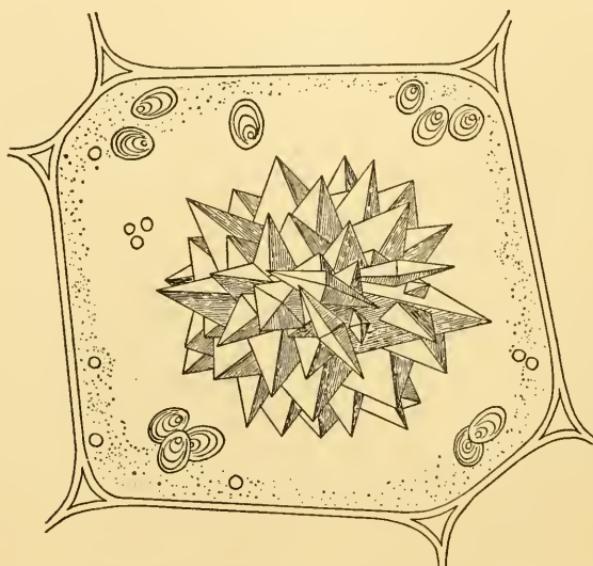


FIG. 11.—Calcium oxalate crystal in a plant cell. (The lamellated structures at the periphery are starch grains.)

segments. These segments are the chromosomes (Fig. 12c). They collect at the center of the cell where they form the *equatorial plate* (Fig. 12d, 10c). While at the equatorial plate the chromosomes are connected by *spindle fibers* to the *poles* of the cell (Fig. 12d). The fibers are still visible at a later stage (Fig. 12f). At a time which is not precisely known in all cases, the chromosomes split longitudinally. Half of each chromosome then moves (Figs. 12e, 10d) or is moved to one end of the cell. Thus does each new daughter cell get part of each of the original chromosomes. At their respective poles in the mother cell, the newly formed daughter chromosomes collect, lose their identity as distinct structures, and fuse to form the nuclei of the two

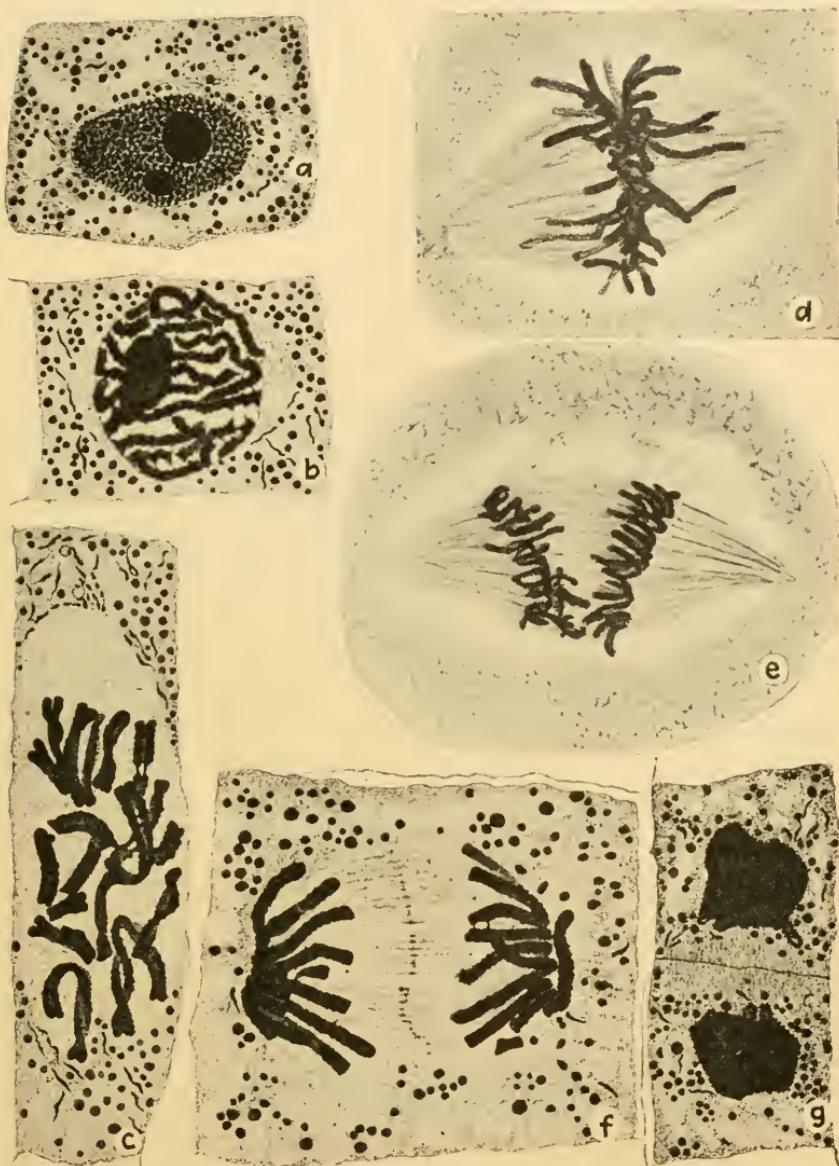


FIG. 12.—*a*, A resting plant cell showing the granular appearance of the chromatin in the nucleus (with two nucleoli) prior to the formation of a thread; *b*, the thread or spireme (with one nucleolus); *c*, breaking of the spireme into individual chromosomes and splitting of the latter (and disappearance of the nucleolus and nuclear membrane); *d*, the equatorial plate; *e*, the chromosomes migrating to their respective poles; *f*, the chromosomes at the poles with spindle fibers thickened at their midpoints; *g*, the forming of the two daughter nuclei and the primitive wall. (*a, b, c, f, g*, *Vicia*, from W. Robyns; *d, e*, *Iris*, from V. Jungers.)

daughter cells (Fig. 12g). This last statement has been tentatively modified by recent work. The chromosomes are supposed to contain centers of hereditary characters, the so-called *genes*, arranged in an orderly and definite manner. If these genes are to keep their respective places, the chromosomes must persist as such in the resting nucleus, otherwise the reorganization of the chromosomes at the next division would probably involve a helter-skelter rearrangement of the genes.

In plant cells, the spindle fibers thicken up at their mid-points (Fig. 12f), forming the *cell plate*, which is the first indication of the new cell wall (Fig. 12g).



FIG. 13.—
Chromosome
“satellites.”



FIG. 14.—Spiral plant chromosomes.
(a from B. P. Kaufmann; b from K.
Sax.)

The manner in which chromosomes get to their respective poles is the subject of much discussion. It has been thought that they are attracted magnetically, that they crawl or worm their way, and that they are pulled by the spindle fibers. This last idea is supported by evidence of a point of attachment of the fiber to the chromosome.

Chromosomes often show peculiarities in external form. They may possess little satellites (Fig. 13).

Recent work on chromosomes has shown them to be of spiral structure. The chromosome was formerly thought to be a fine emulsion of chromatin and other substances, or, what amounts to the same thing, vacuolate in structure, until Kaufmann discovered, or rediscovered, a spiral structure in them (Fig. 14). The spiral structure of chromosomes is now generally accepted as typical of plants, and possibly also of animals, although zoologists have given little attention to this feature of chromosomes.

Studies on the structure of chromosomes by Painter and Metz permit an interpretation of the inner arrangement of parts. Painter has made a general map of the chromosomes of the

salivary glands of *Drosophila*. (These chromosomes are enormous in size and therefore excellent material for study.) He finds that each chromosome has a definite and constant morphology; it is made of segments with bands which entwine the achromatic matrix. The pattern of bands and lines is constant to an extraordinary degree so that it is possible to locate "genes," or loci which stand for definite morphological characters (Fig. 15).

The importance of chromosomes is evident in a number of ways; thus, every organism has a specific number. Man has 48 (female 48, male 47); the fruit fly, 8; and corn, 24. Japanese



FIG. 15.—Chromosome pattern showing loci (bands) of the hereditary determiners or genes. (From T. S. Painter.)

botanists (Kuwada, Morinaga, *et al.*) have been particularly diligent in counting chromosomes and have found that there are 16 in the onion, 12 in rice, 24 in lily, 16 in one species and 24 in another species of crocus, and 30 in tea. The behavior and constitution of chromosomes are among the most fundamental of problems with which the biologist has to deal in his study of living matter. What we are and what we do are determined in great measure by our chromosomes. They carry most, if not all, of our hereditary characters.

D. H. Wenrich discovered anatomical evidence of the individuality of the chromosome; this permits distinguishing them and supports the concept of their continuity from generation to generation. He observed the linear arrangement of particles (*chromomeres*) in them. This is in harmony with the present-day

idea of a linear arrangement of the genes, or hereditary carrying units. He showed also that the chromosomes are paired—a paternal chromosome with a maternal one. These three fundamental concepts are the basis of the modern chromosome theory of heredity.

Certain chromosomes have been singled out as having to do with this or that hereditary factor; for example, it was early noticed that in some animals there is an accessory chromosome. McClung first suggested that the X, or accessory, chromosome is associated with sex, a hypothesis now generally accepted. Proof of this has come from many sides. The accessory chromosome is often of different shape, as in the fruit fly (Fig. 16), or it may be an extra chromosome resembling the others. This latter condition exists in man where the male has 47 and the female 48 chromosomes. The latest evidence indicates that sex is not determined by this one accessory chromosome alone, though it does seem to play a primary part.



FIG. 16.—The four pairs of chromosomes and the accessory chromosome in the male *Drosophila*. (From T. H. Morgan.)

Cell division, or mitosis, proceeds in a similar manner in all cells possessing chromosomes. (These structures are lacking, so it is thought, in the lowest forms of life such as bacteria.) In animal eggs, division is accompanied by the appearance of *asters*, two starlike bodies which form immediately after the sperm enters the egg (Fig. 17).

The *centrosome* is a special body occurring with the asters in animal eggs. It is a minute granule situated at the center of each aster when the cell is in division (Fig. 17). The centrosome has gone through the same vicissitudes in regard to its reality as have most other cell inclusions. Fry declares it to be a coagulation center and not a permanent cell structure, while E. B. Wilson sees in it a cell organ of some significance with genetic continuity. As yet we have no definite knowledge of the function of the centrosome. Centrosomes are present in many lower plants but apparently absent in higher forms.

It is the function rather than the reality of cell parts which troubles us most. R. A. Harper regards all cell inclusions as centers of metabolic activity rather than as cell organs. Thus, the green chloroplast is not so much an organ as a "place where"

a specific activity is carried on. The distinction implies that a cell part, when regarded as a place where, may change its position or disappear entirely, while an anatomical organ must persist.

Ludford points out the importance of cell inclusions in diagnosis. The so-called *virus bodies* are such inclusions which, to some extent at least, differ from the bodies usually occurring in cells. They occur principally in association with disease, mostly those caused by viruses such as vaccinia (cowpox), rabies, measles, scarlet fever, diseases of the fowl, etc. In rabies and fowl pox, the cell inclusions or virus bodies occur with such

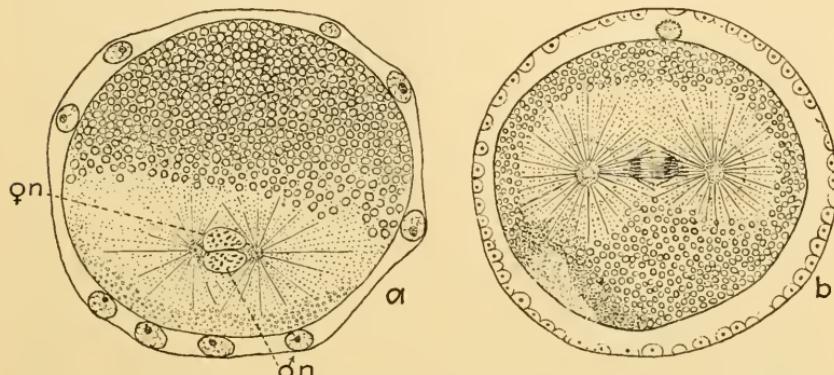


FIG. 17.—*a*, An Ascidian egg just before fusion of the male (σ^n) and female (φn) nuclei, showing the two asters (star-like bodies), in the center of each of which is a centrosome; *b*, cleavage of an animal egg (*Ciona*) showing chromosomes attached to spindle fibers and migrating toward the poles (the centers of the two asters). (From E. G. Conklin.)

constancy and are so characteristic that they are of diagnostic value. The best known among these inclusions are the *Negri bodies*.

Fixation Artifacts.—Most of the cell structures which have been and are about to be described here were first discovered in material prepared, or, as we say, *fixed* (killed with reagents such as alcohol or formaldehyde), and stained. Such cells are dead, and the protoplasm coagulated. There naturally arises the possibility that the structures present are artifacts (*i.e.*, are artificially produced). But as most of these structures have since been seen in the living state, doubt as to their reality no longer exists. A number, however, are still on the doubtful list in the minds of some workers; among these are the centrosomes, Golgi apparatus, and spindle fibers. The existence of spindle fibers is questioned

because they cannot be seen in living material. Whether or not they are real or artifacts is of interest not only in so far as the fibers themselves are concerned but as a critical attitude toward all structures seen in killed and stained material, some of which are certainly artifacts. Spindle fibers are usually to be seen only in fixed material, but as they appear in practically all tissue cells killed with a variety of fixatives, and as they always occur in the same position relative to other cell parts, they must represent something in the living cell. This may be either a preexisting structure of a purely anatomical nature or a field of force which brings about the linear orientation of particles, such as a magnet does to iron filings. Recent work by Bělař supports the reality of spindle fibers. He has followed division in living cells, made constant comparison with cells that have been killed, and finds similar structures, including spindle fibers, in both. These fibers may be made visible in living (dividing) cells if a mild acid is added; they disappear when the solution is again made alkaline; and this may be done without permanent injury to the cell, which continues its normal division. Such behavior supports the cytologist's experience that spindle fibers show up in cells killed with acid fixatives but not when killed with basic ones. Strasburger said if you do not get spindle fibers, add acid (acetic), and you will, and ever since cytologists have been adding acid to their fixatives. The Belgian school of botanists, represented by Gregoir, Jungers, and Robyns, does not regard spindle fibers as real but merely as an indication of an orientation of granules, that is to say, the fibers are not real in themselves, but are the expression of something else which is real. There are others who regard spindle fibers as actual fibers or threads.

The Vacuole.—The vacuole is one of the most important of cell parts. The typical mature plant cell contains a large central vacuole or cavity which is filled with water, salts, sugars, and other substances (Fig. 4). The protoplasm of younger plant cells is devoid of a large central vacuole and contains, instead, many very small vacuoles. It is, therefore, said to be vacuolate. This is probably rather generally true of protoplasm. Dangeard has said that there is no cell without a vacuole. In certain organisms, particularly the Protozoa, the vacuole attains a very special form, with, possibly, distinct functions.

Spallanzani, the same Italian priest who, questioning everything, proved that life comes only from life, first (in 1766) described the *contractile vacuole* of Protozoa (Fig. 25). A contractile, or pulsating, vacuole is not usually found in plants. The animal-like, free-swimming swarm spores of low forms of plant life, such as algae and slime molds, possess contractile vacuoles. For this reason, some of these lowly forms are considered to be of animal rather than of plant origin.

Vacuoles may be classified under the following types:

I. Nonpulsating	II. Pulsating	III. Doubtful forms
A. Simple and not rhythtmical	A. Large central	A. Alveoli
B. Complex and rhythtmical	B. Small and scattered	B. Golgi apparatus
1. Permanent	1. Food	
2. Wandering	2. Tannin	
	3. Lecithin	

The foregoing classification is merely a temporary and convenient grouping to assist in a coherent discussion. The form, structure, contents, mechanism, origin, and function of vacuoles differ greatly. The most common type is globular and non-pulsating. In animal cells, this type of vacuole is small, numerous, and scattered, while in plants, it is usually single, of large size, and assumes a prominent central position in the mature plant cell. The contents of such vacuoles consist of salts, sugars, and nitrogenous (protein) matter in aqueous solution or colloidal dispersion. Many one-celled animals contain a contractile vacuole. It may have a fixed position, as in the more highly organized forms of Protozoa such as Vorticella and Paramecium, or it may be of the wandering type, as in Amoeba. Less common is the reticulate, or netlike, vacuole which has recently been brought to attention through the work of I. W. Bailey (Fig. 18). This type of vacuole is relatively common in plant cells and may possibly be identical with the Golgi apparatus (Fig. 5) in animal

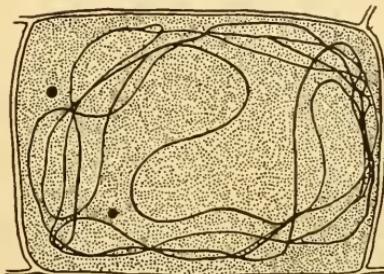


FIG. 18.—The filamentous type of vacuole found in certain plant (pine) cells. (From I. W. Bailey.)

cells. Not only the similarity in external resemblance of the Golgi apparatus and reticulate plant vacuoles but also the fact that silver, used as a fixative, is reduced in both suggests that the two are identical structures.

The filamentous vacuole is a remarkable structure. It may be of such length as to resemble a skein of yarn (Fig. 18). No pioneer worker in cytology would ever have recognized such a structure as a vacuole. Holmgren, in 1903, first described structures, since known as *Holmgren canals*, which are similar to if not identical with filamentous vacuoles. Earlier investigators missed the threadlike plant vacuoles, because these structures show up only when the cell is killed in basic (alkaline) fixatives. Cytological technique has been carried on mostly with acid fixatives, because they are the best for revealing chromosomes on which the interest of biologists has primarily centered. Short, filamentous vacuoles resemble rod-shaped mitochondria, which has led to the statement that there is probably an identity between the two (as in the case of the Golgi apparatus of animals and the reticulate vacuole of plants) but cytologists believe that mitochondria are not vacuoles.

Some vacuoles maintain a definite shape; others change. I. W. Bailey describes threadlike vacuoles as arising from spherical ones through active streaming of the protoplasm. In the change which takes place from the one extreme to the other, the vacuole may, in an intermediate stage, resemble a string of beads. When vacuoles are of maximum size they become huge cavities occupying nearly all of the volume of a cell, only a thin layer of protoplasm remaining.

The contents of vacuoles vary as much as do their shape and size. Probably all typical vacuoles contain salts and sugars in aqueous solution. It is very likely that they also contain some protein matter. Aleuron grains (minute albuminoid granules) have been described as present in some vacuoles, and tannin in others. Certain fatty substances such as lecithin may also be present. More than this cannot be said. It is not always easy to distinguish vacuoles by their contents. I. W. Bailey has shown that two vacuoles within the same plant cell may be identical so far as the human eye can detect, yet one stores tannin and one not.

The function of vacuoles is probably also varied. Some extraordinary uses have been ascribed to them, but the three

most likely ones are the maintenance (in plant cells) and the regulation (in Protozoa) of osmotic (solution) pressure; storage (in plant cells); and excretion. There can be no question that the first of these functions is fulfilled by the large central vacuole of plant cells which maintains the turgidity (osmotic pressure) of cells. In this connection, it is interesting that fresh-water Protozoa possess vacuoles while marine Protozoa do not, but marine Protozoa which have become accustomed to fresh water acquire vacuoles, while fresh-water Protozoa accustomed to salt-water lose their vacuoles. The function of food vacuoles is equally definite. They are formed when food is engulfed by Protozoa. The function customarily ascribed to the contractile vacuole of Protozoa is that of excretion through the discharge of waste products; in other words, the vacuole is crudely comparable to the kidney of higher animals.

Minute protoplasmic *alveoli*, little sacks or cavities which often assume great regularity in form (Fig. 115), are also vacuoles of a kind. C. V. Taylor has suggested this for Euplates. He has seen an alveolus from a disintegrating Euplates float out into the water, maintain its identity while swelling like a true osmotic (solution pressure) system, and ultimately burst.

C. J. Chamberlain has said that all types of vacuolization in plant protoplasm, from the most minute globules to the large central vacuole, are but forms of the same thing. The transformation of spherical vacuoles into threadlike ones is evidence of this. But it is equally true that the contractile vacuole of Protozoa is a structure different, if not in function then at least in its mechanism, from the simple, nonpulsating vacuole typical of protoplasm in general, especially of plant cells.

The Size of Cells.—Cells are mostly microscopic, though some are visible to the naked eye, and some few quite large, relatively. Certain one-celled organisms are large enough to be seen without a lens, though they are then mere specks. Pine cambium cells may be $2,000 \mu$ (2 mm) long; and cells of the alga *Nitella* may be several inches in length (Fig. 33). The one-celled alga *Valonia*, which is spherical in form, may be half an inch in diameter. Cells which build up tissues are usually of microscopic dimensions. The *Elodea* leaf cell shown in Fig. 4 is $\frac{1}{150}$ in. long, about one-quarter as wide, and one-eighth as deep. Expressed in millimeters, such a cell is 0.15 by 0.04 by 0.03 mm. Expressed in

microns (μ) or thousandths of a millimeter, which is the unit of microscopic dimensions, an *Elodea* leaf cell is about 150 by 40 by 30 μ . Its nucleus is 0.010 mm., or 10 μ , in diameter. The cellulose wall is about 0.001 mm., or 1 μ , thick. Among the smallest of cells is the human red corpuscle. It measures 8 μ across, which is 0.008 mm., or 0.0003 inch. It would require 1,500,000 red corpuscles laid side by side to cover the average little finger nail.

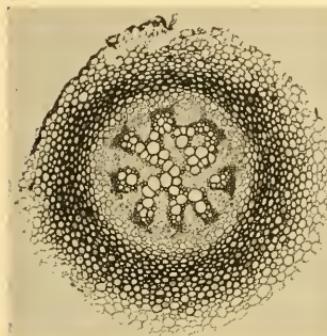


FIG. 19.—Section of a *Lycopodium* rhizome (under-ground stem) showing tissues of several kinds; the large cells in the center are the water-conducting xylem.

mis, or skin; muscle; nerve; cork (Fig. 6); and xylem, or the conducting tissue of plants (Fig. 19). When tissue is young and undifferentiated, it is embryonic. The growing tip of a root or stem is such tissue with the special function to produce cells. It

Tissues.—Cells belong to two main categories, those which form *tissues* and those which live as separate individuals. A tissue is any group of cells of a similar kind, all having (usually) a single, definite function. Such tissues are epider-



FIG. 20.—The growing point of a fern rhizome showing the meristem tissue with its large triangular initial cell (in the center), which is the mother cell of all cells about it. (Figs. 19 and 20 are from G. H. Conant.)

is known as *meristem*, a botanical term indicating actively dividing and perpetual young tissue (Fig. 20).

Nearly two centuries after Robert Hooke discovered cells in cork, the Germans Schleiden and Schwann advanced the *cell*

theory, which states that all living bodies are built of cells, just as a honeycomb is built of chambers formed by partitions of wax. During the World War, some one discovered that not the Germans but a Frenchman, Dutrochet, had first formulated the cell theory, and then, as is so often true, when the matter was looked into, it was found that six other scientists had simultaneously and independently propounded the same theory in one form or another, but the credit is still usually given to Schleiden and Schwann. Indeed, if we are to observe the rule of priority, we must go back to the ancients, for it was Aristotle who, in a sense, first advanced the cell theory, even though he had never seen a cell. He used in its place the hypothetical term "elements." In his "Historia Animalium," he writes "of the parts of animals some of which are 'simple' (elements or cells) and others 'composite'" (tissues). Later, he carried this idea still further when he wrote of "elements," "parts uniform with themselves," and "parts not uniform with themselves," that is to say, cells, tissues, and organs. He undoubtedly did not think of the cell as we know it today, but he had the concept of the organism built of units. The chief facts brought out by the cell theory are that all organisms are built of structural units essentially alike, and that these units, or cells, are themselves complete living entities.

This last concept led to the faulty idea that cells in tissues are isolated units. Cells when acting as parts of larger bodies do not live and function wholly independently of each other. It is not enough for a cell to go on living its own independent life; it has its duty to other cells and to the organism as a whole, just as has a citizen to the state. No collective whole, be it a community, a machine, or an organism, can function unless its structural units are coordinated. Each cell influences and is itself influenced by the activities of every other cell in the organism. Equally important to this interrelationship between cells within the body is the influence of the extracellular environment. Cells are bathed in body fluids. It was the great French physiologist Claude Bernard who emphasized the importance of the inner environment of the cell.

The question arises whether the body fluids are alone sufficient to maintain a coordinated activity between cells. Is not some *living* connection between cells necessary? In animals, the

nerve fibers serve as lines of communication between tissues. More significant is the fact that animal cells are in direct contact with one another; protoplasm touches protoplasm (the intervening cell membranes are of protoplasm and therefore do not nullify our statement). But plant cells are separated by heavy cellulose walls. If a tip of the leaf of the sensitive plant *Mimosa pudica* is touched, within a few seconds all the leaflets are closed, and the petiole drops. Communication has taken place from cell to cell. One can imagine several ways in which this might happen; for example, an electrical stimulus may have traversed the electrolytic (salt) solution which bathes the cells and per-



FIG. 21.—Protoplasmic connections (plasmodesmata) between the cells of, *a*, the persimmon, *Diospyros* (from C. J. Chamberlain); *b*, the moss *Madotheca* (from B. Němec).

meates the cellulose walls. But such a nonliving method of communication would not seem to be very satisfactory. One would suspect the presence of a vital, a protoplasmic connection of some sort. A very careful examination shows that such a connection does exist between cells. Vital contact between plant tissues may be maintained by delicate intercellular protoplasmic strands known as *plasmodesmata* (Fig. 21) which penetrate the heavy cellulose walls. How general these are in plants is unknown; indeed, their very existence is doubted. The presumed protoplasmic strands are said by some to be but pits or canals in the cellulose wall, but L. G. Livingston supports the earlier work of Němec and makes it quite clear that plasmodesmata do exist. Comparable intercellular connections of large size indisputably exist through the sieve plate of sieve tubes (food-conducting vessels). Similar protoplasmic bridges occur in the red algae. These give vital continuity between cells for the transmission of stimuli. To know that protoplasmic connections between cells exist in certain cases is of little help when we learn that apparently it is not so necessary as it would seem. Boysen-

Jensen and a number of others have shown that a stimulus may be transmitted down a growing root and through an intervening layer of gelatin after the tip of the root has been cut off and replaced, with gelatin between it and the root proper. The upper part of the root is subjected to a light stimulus, and the lower part on the other side of the gelatin layer responds by curved growth. The tip of a foreign root may be attached (an oak root cemented on with gelatin to a decapitated wheat root), and response obtained. (Excitation is not transmitted through an intervening layer of mica or tinfoil.) The stimulus cannot be vital in the strict sense. It may be electrical, or a diffusion wave, traveling in a salt solution. Such experiments are interesting and instructive, but they do not disprove the hypothesis that while some vital processes, such as that of stimulus transmission, may be quite simple physical ones, others are of such complexity that they can take place only in living protoplasm. It is our task to distinguish the one from the other.

Cell Types.—The cells that we have so far discussed are among the usual ones of tissues, but there are many other types. Some unique ones occur as parts of tissues, and other extraordinary ones are complete organisms in themselves. Of the unique types of tissue cells the following are a few. Root hairs, so important in the life of plants, are elongated epidermal cells. On the surface of the leaf of the nettle are slender hairs consisting of a single cell filled with a poisonous sap. The wall is thin near the tip, so that if the hair is pressed upon, it breaks, leaving a sharp point. The cell thus acts like a hypodermic needle. In the pith of the rush *Juncus*, the cells are starlike, providing abundant air space. Thus does the shape of a cell suit the particular function to be performed.

Plant cells having specially developed walls are common. The tracheids, which carry water in the stem of the pine, have communicating bordered pits in their walls. Cells of the epidermis of leaves develop an enormously thick outer wall impregnated with waxy cutin. "Stone" cells with remarkably heavy walls are common in plant tissues. The cells in the seed of the date store food in their walls for the young seedling, which later digests it when the seed germinates.

Cells in the animal body may be as varied. The cells lining air passages are covered with cilia, or little hairlike whips. Nerve

cells may be greatly elongated, developing a branching, almost treelike structure (Fig. 22). The cells of bone, connective tissue, and cartilage secrete and embed themselves in a nonliving substance just as the protoplast of the plant cell secretes its cellulose wall.

Unicellular Organisms.—We have so far had to do with cells that are part of a larger living body. There are myriads of different types of cells which live alone. Microscopic organisms constitute a world of their own. Among the more lowly of one-

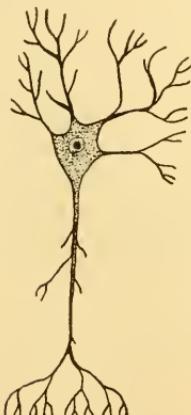


FIG. 22.—Nerve cell. (After L. W. Sharp.)

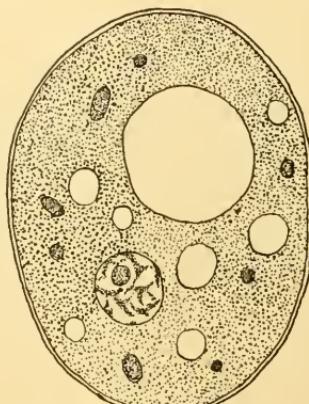


FIG. 23.—A yeast cell. (After Smith.)

celled organisms are those which cannot be definitely regarded as either plant or animal. The bacteria are such cells. The botanist speaks of bacterial *flora*, and the zoologist of a bacterial *fauna* (*flora* is now mostly used). Similarities in the mode of living of bacteria and fungi suggest the plantlike character of the former.

Bacteria are too small to permit a detailed study of their structure. Some are barely visible with the highest power of the microscope. They can, therefore, be classified only crudely on the basis of their external form. Bacteria occur as spheres, rods, and spirals. More satisfying and detailed is a physiological and pathological classification, but this involves a knowledge of medicine, hygiene, plant disease, fermentation, soil science, etc.

Slightly higher in the evolutionary scale is yeast (Fig. 23), another unicellular plant. Euglena is a one-celled, border-line

"plant animal" which the botanist puts under the plant division Flagellata (plants provided with flagellae for swimming), while the zoologist regards it as a Protozoan (or "primitive animal"). The fact that Euglena contains chlorophyll, the green pigment of plants, suggests a plant origin, but the fact that it swims indicates animal characteristics. The botanist accepts this latter deduction and leaves Euglena for the zoologist. But it makes little difference. On the contrary, it is to be expected that border-line organisms exist. If plants and animals arose from a common ancestor, it is only natural that primitive forms neither distinctly plant nor distinctly animal should remain.

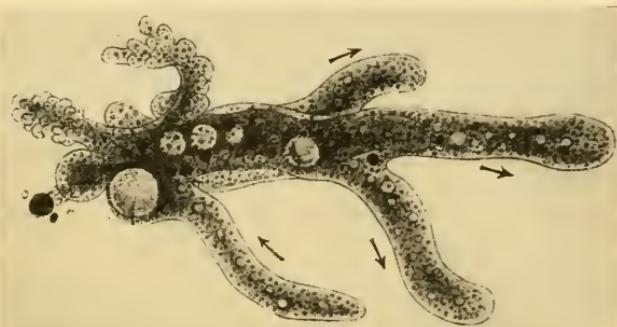


FIG. 24.—*Amoeba proteus*. (From J. Leidy.)

All of the many kinds of one-celled plants and animals are classed together as Protista. Formerly they were referred to, but now only in a popular sense, as "animalculae." The animals among them are known as Protozoa. The most renowned of the Protozoa is Amoeba (Fig. 24). This unicellular animal has become well known to the layman in such expressions as "from amoeba to man," which suggests that Amoeba is the most primitive of living beings. As generally illustrated, Amoeba is shown as a lobed body with a spherical nucleus, a large contractile vacuole, several food vacuoles, and many protoplasmic granules. S. O. Mast has observed far greater details. His drawing of *Amoeba proteus* is so clear that no further description is needed (Fig. 25).

Some indication of how multifarious are the varieties of Protista, or one-celled plants and animals, is to be had from a mere listing of a few of them. A familiar form is the green alga

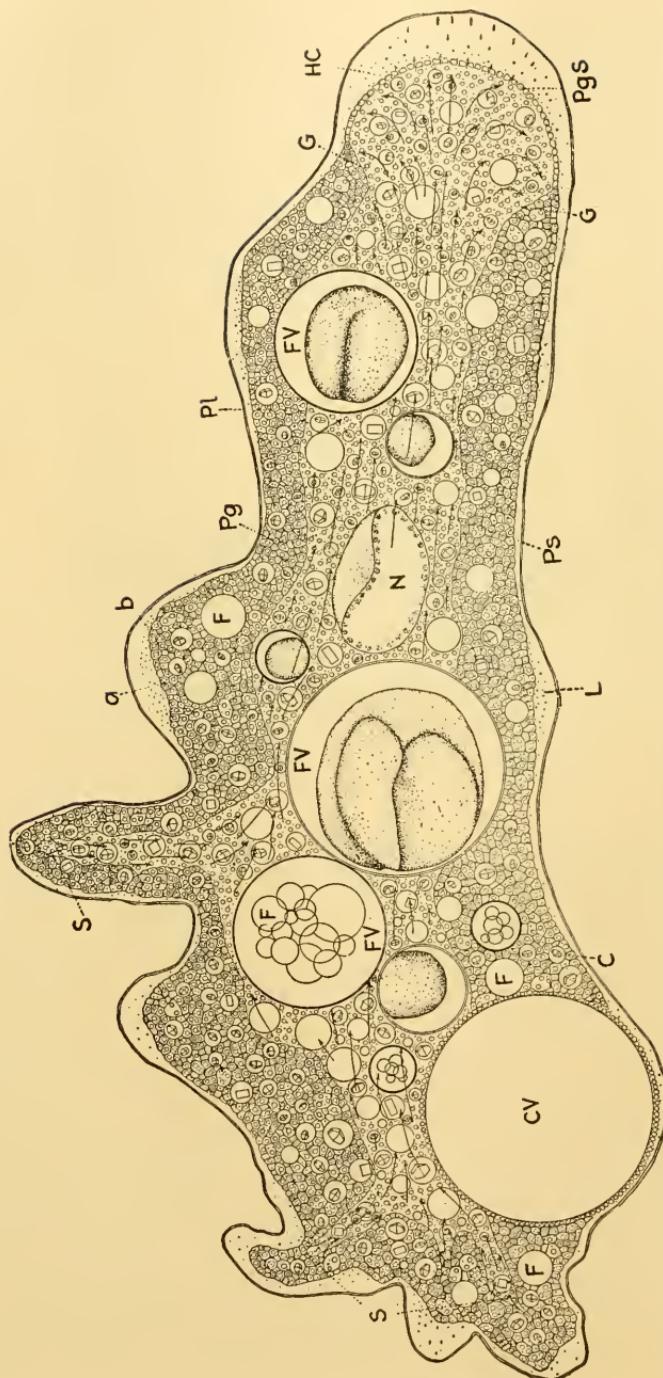


FIG. 25.—Sketch of a horizontal optical section of *Amoeba proteus*. *a*, alpha granules; *b*, beta granules; *P_s*, plasmalsos; *P_g*, plasmagel; *Pl*, plasmalemma; *HC*, hyaline cap; *P_{gs}*, plasmagel sheet; *L*, liquid layer; *S*, region of solation; *G*, region of gelation; *N*, nucleus; *FV*, food vacuole; *C*, crystals in vacuoles; *F*, spherical masses of substance formed in the food vacuoles; arrows, direction and relative rate of flow. (From S. O. Mast.)

Protococcus, which covers the north side of trees and stone walls with a green coating of thousands of cells. The one-celled plants, known as *diatoms*, are very abundant, inhabiting all bodies of

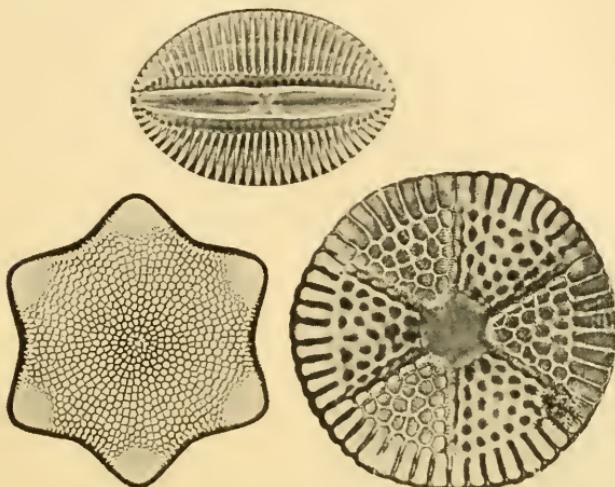


FIG. 26.—Diatoms. (From A. S. Mann.)

water, in particular the sea. They are among the most exquisite of Nature's handiwork, being often beautifully patterned (Fig. 26). Vying with the diatoms for beauty in the world of microscopic organisms are the unicellular animals Foraminifera and Radiolaria (Fig. 27), also inhabitants, like the diatoms, of the first few inches of the surface of the sea. The minute plants and animals that live in the surface of the sea are known as the plankton. The Radiolaria and Foraminifera possess a shell, as do the diatoms, and, because of it, have produced large deposits such as the chalk cliffs of Dover. The siliceous shells of the diatoms constitute the deposits which form diatomaceous earth. The shells of diatoms and Radiolaria are of silica; the shell of Foraminifera, of lime. These creatures differ much

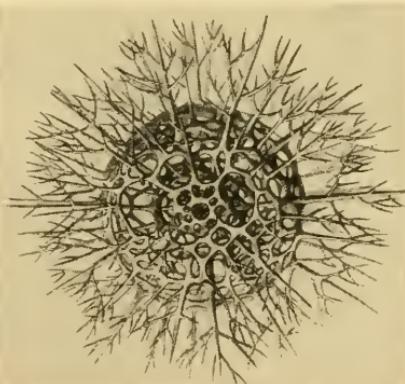


FIG. 27.—*Cromyodrymus abietinus*. (From "Voyage of the Challenger.")

in appearance and habit from naked masses of protoplasm like Amoeba, though all are one-celled organisms.

Among the distinguishing characteristics of cells that are unicellular organisms and those that are parts of tissues is the ability of the former to live singly, while the latter are unable to do so. As a result, the one-celled organisms are often much more complex than are tissue cells and to such an extent that while we are forced to recognize them as single cells, they cease

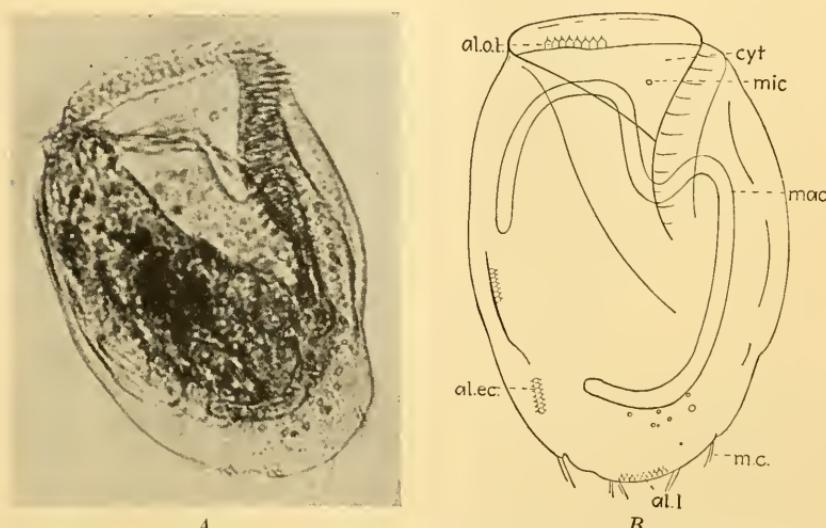


FIG. 28.—*A*, Euplotes; *B*, diagram of Euplotes showing, *mac.* the macronucleus, *mic.* the micronucleus, *m. c.* cirri, *al.* alveoli, *cyt.* the cytostome.

to be so in the strict sense of the word. They are no longer just cells but organisms as well. The protozoan Euplotes illustrates this fact (Fig. 28). This one-celled animal lives in water, as do most unicellular organisms. Euplotes is barely visible to the naked eye as a tiny spot (the actual size is about $150\ \mu$). It is very active, for at various points protrude cilia used for swimming. Some of these cilia are united to form stronger cirri on which the organism can actually walk. The anterior portion is mostly "mouth" into which food, consisting of smaller one-celled plants and animals, is directed by means of other minute cilia; there is also a posterior flange. The interior contains a central region, or "stomach," a worm-shaped macronucleus, a

very small micronucleus, and a rather intricate "neuromotor" apparatus, which is a nervous system in miniature (Fig. 28B). Euplotes is a highly organized individual though but a single cell. It is an organism because it lives alone; it is a cell because it is all included within a single membrane.

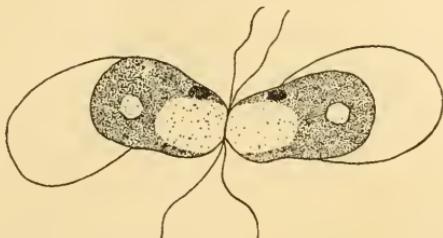


FIG. 29.—The fusion (conjugation) of two *Chlamydomonas*. (After Smith.)

All the tissue cells so far mentioned are vegetative, or *somatic*, ones; that is to say, they are cells concerned in building up and maintaining the plant or animal body in its purely vegetative existence, in growth and in nutrition. Besides the business of living, every organism is concerned with perpetuating its kind. In higher forms of life, certain cells are set aside solely for reproduction. These are the highly specialized cells. In Protista, there is naturally no such division of labor, for the organism is but a single cell. One-celled plants and animals carry on a vegetative life today and tomorrow turn their attention to multiplication, which is usually accomplished by the simple nonsexual method of fission, or pinching in two. Occasionally, a primitive form of sexual reproduction takes place among the Protista; two entire and similar organisms come together, unite by fusion, and later divide (Fig. 29).

In the higher plants and animals, definite cells are formed for reproduction. These cells are the eggs and sperm. Egg cells

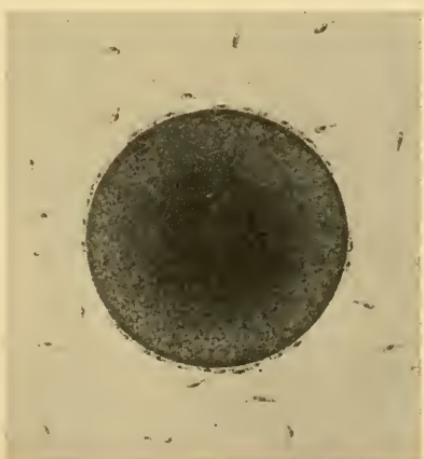


FIG. 30.—An egg of the seaweed *Fucus* surrounded by sperm preliminary to fertilization. (From G. A. Thuret.)

are much alike throughout the living world, whether in a starfish, a fern, or a mammal. They are relatively large, nonmotile, with abundant protoplasm and considerable stored food (Fig. 30). Male sexual cells are quite specialized.

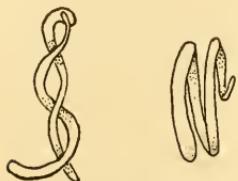


FIG. 31.—Male sexual cells (gametes or spermatozoids) of the liverwort *Pallavicinia*.

In lowly plants and in practically all Metazoa (multicellular animals), the sperm are small, active cells, with very little protoplasm surrounding the nucleus (Figs. 30, 31, 32). The cycads and the closely related Gingko tree are the highest members of the plant kingdom to possess motile male cells. Most of the lower plants (algae, mosses, ferns, etc.) have motile (swimming) male sexual cells, but in higher plants the male gamete, or sex cell, has lost

the property of motility and is carried by the pollen tube to the egg in the base of the flower where fusion of the two sex nuclei takes place. The fusion of the male nucleus with the female nucleus constitutes fertilization.

Unusual Primitive Organisms.—Certain lowly forms of living matter consist of protoplasm with many nuclei, housed in bodies of macroscopic size. Such *multinucleate* bodies may be termed noncellular. They cannot be regarded as single cells in the strict sense, for they contain many nuclei; nor are they tissue, for there are no cross walls. They are cells in so far as they are enclosed in one wall or membrane. The *plasmodium* (protoplasmic body) of the slime molds is a structure of this kind (Fig. 1). The botanists claim the slime molds as plants and call them Myxomycetes ("slime fungi"); while the zoologists claim them as Myctozoa ("fungus animals"). The plasmodium, or body, of slime molds is a naked mass of protoplasm often attaining an area equal to the size of one's hand but exceedingly thin. It is a primitive mass of almost wholly undifferentiated protoplasm like an amoeba except for its many nuclei. One can imagine a thousand amoebae fusing to form a single, multinuclear, undivided mass of protoplasm; indeed, in just such a way may the myxomycete plasmodium actually arise. The "amoebae" are called *myxamoebae* and are amoeba-like, swimming spores which germinate from the spores of the



FIG. 32.—Sperm of the chicken.

parent plant. Slime molds live in decayed wood and in order to reproduce come to the surface, where they form fruiting bodies containing the spores, which later germinate. These latter swim about awhile as myxamoebae and then possibly fuse or individually grow into the myxamoebae or plasmodia, which are the bodies of the plants.

In comparing the plasmodium of a myxomycete with the body of an amoeba, we distinguish between them in that the one is multinucleate and the other uninucleate, yet Vonmiller has described an amoeba with 12 nuclei. Here, as in all our attempts to define and classify, Nature mocks our categories. She seems at times to have had a clear-cut and carefully thought-out plan according to which life forms have developed, but there are examples, especially of primitive organisms, which do not appear to fit into any scheme.

Built somewhat on the same principle as myxomycetes, in that they possess a noncellular, multinucleate body, are the *coenocytic* plants. To this type belong bread mold and the green alga *Vaucheria*. Both consist of a long tube, or *coenocyte*, containing many nuclei but no crosswalls. Similar in structure are the alga *Cladophora* and the stonewort *Nitella* the individual cells of which contain many nuclei and may reach 6 in. in length (Fig. 33). *Valonia* and its cousin *Halicystis* are also large, multinucleate, noncellular bodies. Both are relatively huge (1 to 4 cm.) spheres consisting of a large central vacuole with as much as 50 cc. of sap, enclosed by a thin layer of protoplasm.

Not only are cells so constituted chemically as to carry on their specific tasks, whether that of making food or that of digesting it, but they are often equally well fitted in a purely mechanical way. Examples such as heavy-walled cells, spirally thickened ones, mechanical bracings, and other structural features no less remarkable and perfect from the point of view of engineering are familiar to the plant anatomist. A most interesting case of a pure physical adaptation is that of the cells of the protonema (filament) of

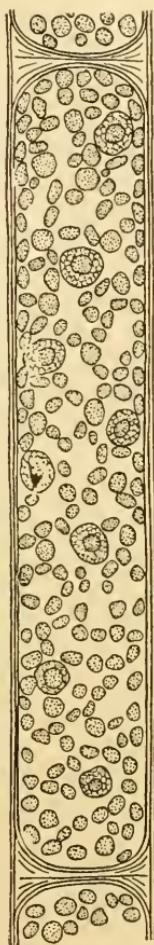


FIG. 33.—
A cell of *Nitella*.

the moss *Schistostega osmundacea* (Fig. 34). This small moss lives in the deep recesses of rocks, usually in caves where the light is weak. Just why it "chooses" such dark places cannot be said, but since it apparently does "prefer" them, it is fortunate that its cells are such excellent optical systems as to collect the feeble light and focus it on the chloroplasts. Each cell of the protonema is a

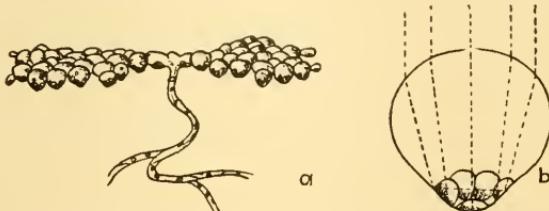


FIG. 34.—*Schistostega osmundacea*; *a*, the entire moss, *b*, a single cell.

lens at the base of which the few chloroplasts are situated (Fig. 34*b*). Light enters at the top and is focused on the chloroplasts, thus concentrating the little sunlight available for photosynthesis. The light is totally reflected after passing through the chloroplasts and thus acquires a green phosphorescent tinge, so that the moss, when seen deep in the moist crevice of a rock, resembles the eyes of a cat at night.

Such are cells; with these and the matter that fills them does this book deal.

CHAPTER III

MODEL MAKING

Lord Kelvin said, "Can ye make a model of it? For if ye can, ye understand it, and if ye canna, ye dinna!" This remark epitomizes the philosophy of science in Mid-Victorian days. There is much to be said in favor of visualizing an idea by making a mental, if not an actual, working model of it, but a model is by no means necessary in order to comprehend an idea; and, on the other hand, while a model may greatly elucidate an idea, it may also obscure it and lead to a wholly erroneous conclusion. The fact that the model works very well and does precisely what it is intended to do is not evidence that the mechanism which it is supposed to imitate also works that way. There was once constructed a model intended to show how the contractile vacuole of a protozoan operates. The model contained valves and numerous other appliances. It worked and did what the contractile vacuole does, but few would go so far as to say that a contractile vacuole contains mechanical devices such as the model contained or anything like them.

Model making in biology includes any attempt to imitate vital phenomena with nonliving material. It is more extensively practiced than one, on first thought, realizes. The experiments of Bütschli, in which he produced structure in gels similar to those in protoplasm; the experiments of Rhumbler, in which the movements of liquid drops of mercury were observed with the hope that they would throw some light on the mechanism of amoeboid movement; and the experiments of R. Lillie with iron wire immersed in solution, imitating the action of nerves, are all experiments in model making.

A number of common laboratory procedures imitate happenings in the living world accurately in principle, without, however, reproducing the vital processes exactly as to material and form. Osmotic cells made in the laboratory function just as do plant cells, even though in construction or material they differ from the living cell.

The sudden changes in protoplasmic consistency, from quite liquid to very firm, have been compared to the thixotropic behavior of gels (page 150). While no specially constructed model is here necessary, yet any gel which also goes through these changes becomes for the moment a model.

Freundlich has observed spindle-shaped figures in concentrated vanadium pentoxide and benzopurpurin which he believes may be formed in a manner similar to the spindle of a dividing cell (page 20). The former when made to reproduce the latter is a model of it.

A number of the properties of protoplasm, such as its elasticity, may be duplicated in nonliving matter, particularly gels, which thus become models of the living substance in so far as they imitate one quality.

Imitations of living cells as a whole have often been attempted. Among the first of these were the experiments of Berthold, in imitation of the movements of amoebae. The experiments of the Mexican Herrera include imitations of many cellular activities. By placing drops of caustic soda stained with rhodamine in a mixture of 1 part olive oil, 1 part resin, and 40 parts gasoline, Herrera produced what he called "colpoids," which are imitation amoebae. They show internal flow as well as body movements, due to changes in surface tension and the repulsive action of osmotic currents. A number of other reactions occur in these colpoids in imitation of vital processes, such as conjugation (the fusion of two individuals), deformation, contraction of vacuoles, formation of pseudopodia, division, growth, and the phagocytosis, or ingestion, of carbon particles impregnated with acetic acid. This last reaction is in imitation of the taking in of food by Amoeba. A more familiar experiment of this kind is that of Rhumbler, who showed that a drop of chloroform under water will not take in a piece of glass, but if the glass is coated with shellac it is immediately taken in. After "digesting" (dissolving off) the shellac, the chloroform ejects the glass. A living amoeba will ingest particles which are not good as food—particles such as those of the dye carmine—and immediately give them up. The three phenomena, a living amoeba eating real food (organic matter), a living amoeba eating imitation food (carmine), and an imitation amoeba (colpoid) "eating" imitation food (carbon) may all be due to the same force, *viz.*, a change in surface tension.

If true, then, in what way does the act of the living cell differ from that of the model?

Herrera and Leduc have made imitation cells with nuclei and mitotic figures.

A study by Kuwada of the distribution of magnets floating on water in an attempt to explain the orientation of chromosomes in cells is an experiment in model making.

One of the most fundamental and much studied problems in biology is the mechanism of cell permeability. The manner in which the protoplasmic membrane lets certain substances pass and others not and certain ones enter in greater quantity than others is unknown. There have been many attempts to emulate the permeability of the plasma membrane. Osterhout and Northrop have made artificial cells which imitate the permeability of the living plasma membrane quite well. Northrop constructed thimbles of collodion membranes and found them to be permeable to water, ammonia, and hydrochloric acid; slightly permeable to carbon dioxide, oxygen, hydrogen, and weak acids; and practically impermeable to salts, strong acids, bases, sugar, and glycerin. The permeability resembles that of living cells.

Another more pretentious type of artificial plant cell is that of MacDougal and Moravek. Thimbles of porous clay or cellulose were impregnated with agar, then dipped in pectin, in imitation of the root-hair cell with its pectin layer in the wall. The inner surface of the wall of the artificial cell was coated with fatty substances (lecithin and cholesterol), and, finally, a layer of "protoplasm" was added, *i.e.*, of agar, or gelatin, and proteins. Diversity in results was obtained by variations in the construction of the cell. One cell, with a lecithin layer, permitted the entrance of sodium chloride, potassium chloride, and calcium chloride, so that permeability was greater for sodium, less for potassium, and least for calcium, thus imitating the selective permeability of the living cell. Omission of the fatty layer and the use of certain proportions of gelatin and agar reversed the relations between the rates of entrance of sodium and of potassium. The entrance of ions into the artificial cell shows a parallelism to that in the living cell which is very striking. The reactions of the artificial cell to salt and to acidity changes also fairly well duplicate the swelling of living tissues of cacti in similar solutions.

Shall we ever be able to make a *living* cell? An answer to this question is impossible. While some men of scientific standing believe the synthesis of living matter to be possible, others regard it as very unlikely; in any case, it has as yet not been accomplished. The work of Pasteur proved that spontaneous generation does not (ordinarily) exist and that organisms arise only from parents, from living antecedents. This fact gave rise to the aphorism "Omne vivum ex ovo," which has since dominated all scientific and philosophic thought. The pre-Pasteur "scientific" formulas for producing life are no longer taken seriously; on the other hand, those scientists who believe that the bridge between the living and the nonliving is not very great tacitly admit that it may be possible to cross this bridge some day.

In justice to the model makers, it should again be emphasized that they do not assume that their imitations are alive. Model making teaches much and may approach the actual state of affairs in living matter. Imitations of life processes should be viewed not necessarily with skepticism but with the full realization that they *are* imitations and likely to differ greatly from the way living matter performs the same function, even though both accomplish the same result. On the other hand, it is important to realize that, as Leduc says, "considering the impossibility of defining the exact line of demarcation between animate and inanimate matter, it is astonishing to find so much stress laid on the supposed fundamental difference between vital and nonvital phenomena."

Whatever protoplasm and life may be, their mechanisms are physical and chemical, and these mechanisms can be studied independently. They represent certain laws, and the laws are true even if, in a living system, hundreds of processes go on at the same time. Model making is simply an attempt to analyze the laws of protoplasmic behavior.

CHAPTER IV

MICRURGY

The microdissection of living cells was first done a century ago. The pioneer workers on living matter, von Mohl, Dujardin, Johannes Müller, and von Baer, dissected cells with great ingenuity. Purkinje (1844) suggested microdissection, and Chabry (1877) carried it out. Later came the micromanipulative work of W. Roux, E. B. Wilson, J. Loeb, and others, which had to do with relatively simple dissections, involving such operations as the puncturing of an egg and the separation of the cells (blastomeres) of an embryo. H. D. Schmidt as early as 1859, Herlitzka in 1895, Kopsch in 1900, and McClendon in 1905 carried on microdissection with mechanically controlled needles, but the work ended with their few experiments. It was not from the work of the pioneer microdissectionists that *micrurgy* directly arose to its present state of perfection.

The task of obtaining pure cultures is one with which the bacteriologist has always had to deal. The condition toward which he strives is a pure culture started from a single bacterium, for then not only is

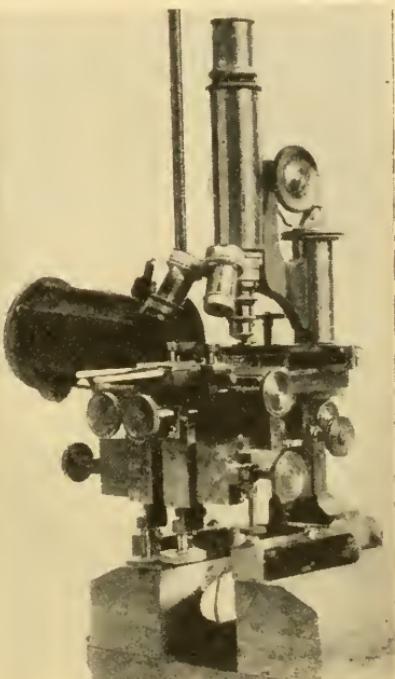


FIG. 35.—The Barber-Kite microdissection instrument. In this photograph, the instrument is equipped with an electromagnet for attracting minute nickel particles which have been inserted into the living cell, and which, when attracted, give an indication of the viscosity and the elasticity of the protoplasm. The two fine glass needle points and the heavier tip of the magnet core are to be seen within the faintly visible glass chamber under the microscope lens.

the colony pure, but it represents the progeny of a single parent. To attain this, Schouten in Holland (1899) and Barber in America (1904) devised apparatus to hold and control the movement of needles and pipettes. Schouten's apparatus consists of heavy bars held together by a steel spring. A thumbscrew presses against one bar, separating it from the other. In this manner, movement of the needle is obtained. As Schouten's work involved the isolation of bacteria or Protozoa, he devised a method for catching a microscopic organism within a film of liquid formed by the loop of a fine glass thread when dipped into a solution.

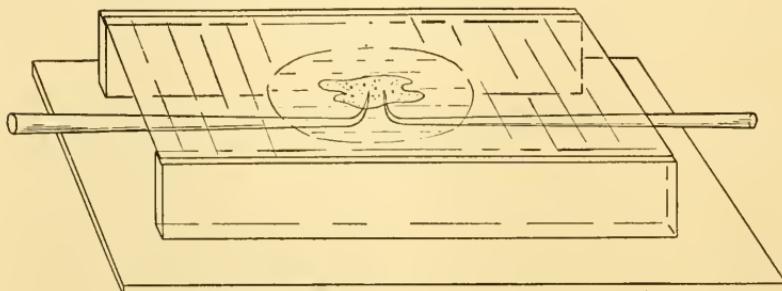


FIG. 36.—Moist chamber for micromanipulative work.

The Barber pipette holder in its original design (Fig. 35 is a modified form of it) consisted of a support built of three parts, each sliding upon and at right angles to the other, and each controlled by three screws. The instrument was fastened near or directly upon the microscope stage. On its top was a clamp to hold a pipette. The latter projected into a small glass moist chamber where bacteria were suspended in a hanging drop. The three movements, horizontal, lateral, and vertical, which were imparted to the holder, made it possible to bring the pipette into any position in space.

The isolation of a single bacterium proceeds as follows: A drop of the culture solution containing bacteria is placed on a glass cover slip. The slip is inverted and put into position as the roof of a moist chamber (Fig. 36). A single bacterium is sought through the microscope. The mouth of the micro-pipette is then brought into position directly under the bacterium and slowly raised until the organism is drawn into the pipette with a small quantity of the solution, either by the

capillary attraction of the pipette or by suction produced by a bulb or syringe attached to it. The cover of the moist chamber is now replaced by another containing pure culture medium into which the single isolated bacterium is ejected from the pipette.

Barber occasionally substituted a needle for the pipette and indulged in some simple experiments in dissection, such as pulling an amoeba apart. He thus saw the possibilities of his instrument for microdissection. It was, however, Kite who converted the Barber pipette holder into a microdissector and used it for this purpose. In so doing, he inaugurated a new method of microscopical technique, which, chiefly in the hands of Chambers in America and Péterfi in Germany has done much and promises more toward an understanding of the anatomy and physiology of cells. To this new field of biological endeavor Péterfi gave the name *Mikrurgie* (*micro*, small; *ergon*, work).

Types of Instruments.—With the statement that several other less well-known instruments have been made, we can limit ourselves to six types of micromanipulators, the two original ones of Schouten and Barber and the four later models of Chambers, Péterfi, Taylor, and de Fonbrune.

Kite modified the Barber pipette holder by joining two holders so as to form one double instrument (Fig. 35), which he attached directly to the stage of the microscope.

The chief fault of the Barber-Kite instrument was loose play (lost motion) in the threads and consequent lack of rigidity. Great precision in movement is most essential when dissecting under the highest power of the microscope. To overcome this defect Chambers designed an instrument based on principles somewhat similar to those of Schouten. Heavy steel springs are banded together at one end and forced apart by thumbscrews at the other end. The springs are fastened to an upright pillar. Movement of the needle is obtained by forcing the springs apart.

The Péterfi (Fig. 37) micromanipulator is of excellent construction, and the Taylor model a very solid instrument. Both have the great advantage of freedom and extent of movement. The needles can be inserted in their holders away from the moist chamber and then brought into position with mechanical control.

Among the newer models, that of P. de Fonbrune is the most ingenious (Fig. 38). It consists of a universal joint which controls three pistons operating against air. By means of three tubes, the pressure exerted by the pistons is conveyed to a separate machine where three metal diaphragms, similar to those of aneroid barometers, are forced out or drawn in and thus control, by means of levers, the rod to which the

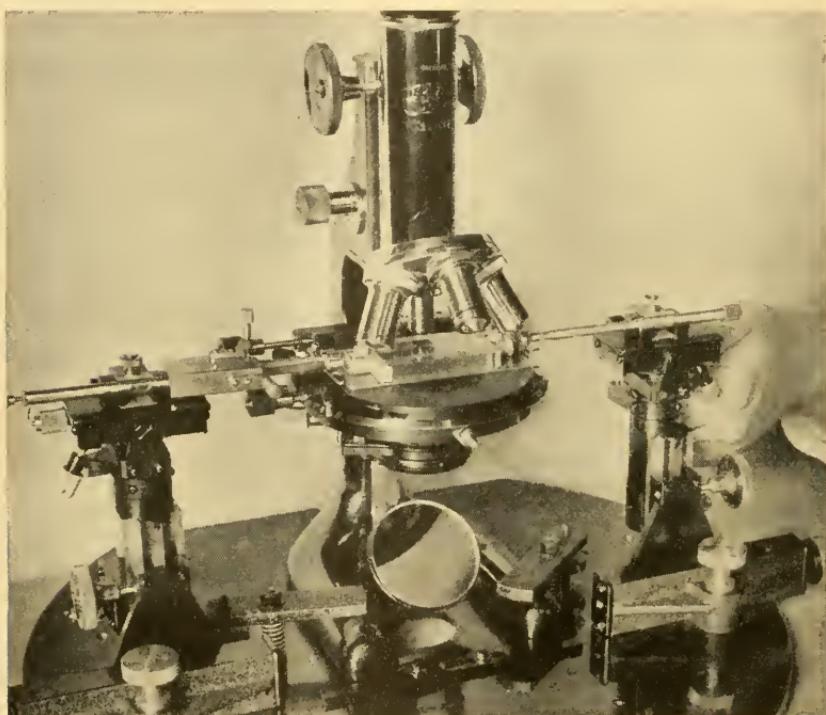


FIG. 37.—The Péterfi micromanipulator. (*From Carl Zeiss.*)

microneedle or pipette is clamped (Fig. 38B). The manipulator operates with remarkable precision, being wholly free from lost motion. The independence of operating and receiving mechanism eliminates vibration. Additional advantages are that the hand of the operator and the needle point move in the same direction and that the operator's hand need never leave the one lever with which all movements are performed. De Fonbrune has also constructed an instrument for automatically drawing needles and pipettes under the microscope lens.

Technique.—The micrurgist must himself prepare most of the tools to be used with the micromanipulator. Certain of them can be bought, some of which are useful and timesaving,

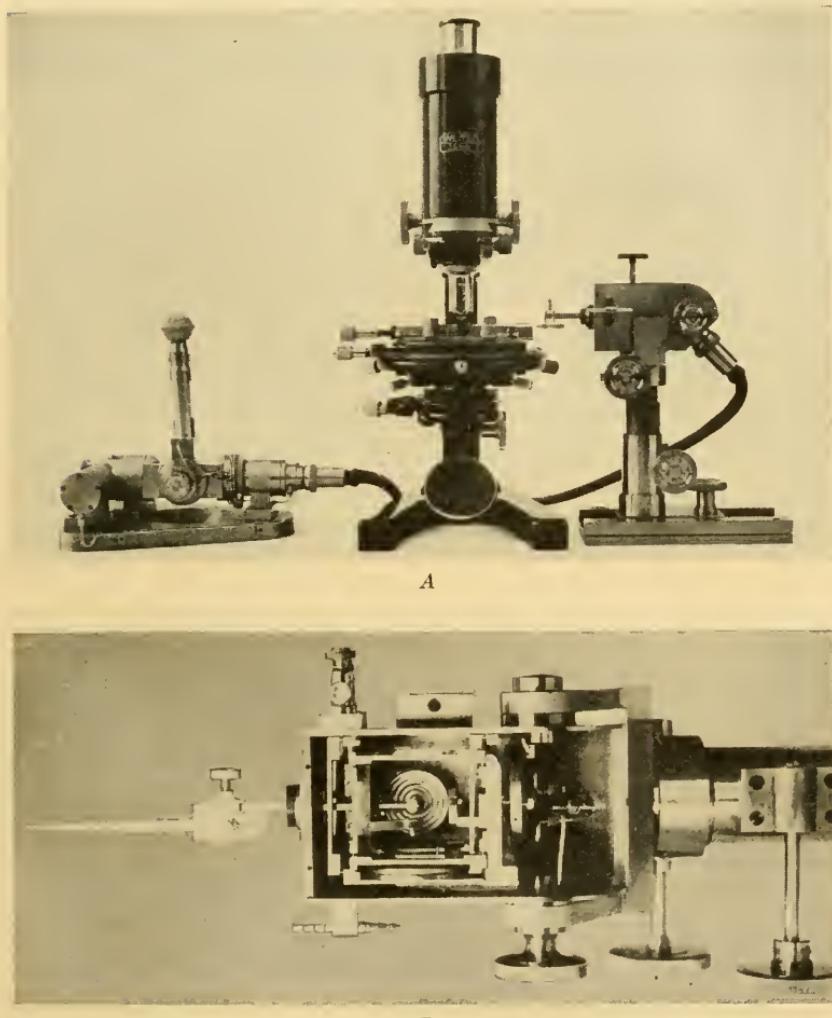


FIG. 38.—A. The de Fonbrune micromanipulator; B, detail of the de Fonbrune micromanipulator showing the aneroid diaphragms and needle.

while others are better made in the laboratory. The first accessory to be constructed is the moist chamber. One may purchase a very neat metal and glass one, but just as satisfactory is a chamber made from a large (5 by 8 cm.) microscope

slide on which are cemented (with balsam) two glass bars 5 cm. long, 8 to 10 mm. high, and 2 to 4 mm. thick, placed slightly less than 24 mm. (the width of a cover slip) apart (Fig. 36). The two needles may enter at one and the same end, as in the old Barber-Kite or the Chambers apparatus; or at both ends, as in the Taylor and Péterfi instruments. The chamber is held in a mechanical stage, which is used almost as much in the process of dissection as are the needles, since it is often more convenient to grasp the material with a needle and then move it by means of the mechanical stage than to move the needle.

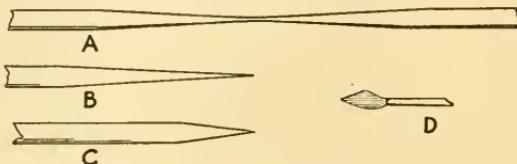


FIG. 39.—*A*, Glass rod preliminary to making a needle; *B*, *C*, types of needles; *D*, micro-spatula.

The next task of the micrurgist is to make his needles, and this is the most difficult part of the technique for a beginner, though it does not long remain so for those who have patience and some dexterity. The needles may be made of glass tubing or rod. The latter is better, as a broken tip leaves the needle point still usable.

Hard (Jena) glass is usually preferable to soft glass but not necessary for ordinary work. Quartz may be used, giving very tough points, but it requires intense heat to draw it and, except for special work, is not worth the trouble. The rod should be three or four millimeters in diameter. It is first drawn in the flame of an ordinary Bunsen burner to the shape shown in Fig. 39A; next, the thin central region is drawn still finer in the very minute flame of a microburner; the finely drawn portion is now held near (not in) the microflame until the right degree of softness (a dull red color) is reached. When sufficiently soft, the thin rod will give way under the pull of the hand; it must be quickly drawn so as to form a rapidly tapering point (Fig. 39B). A good needle should not be over 1μ at the tip and should taper correctly so as not to be too flexible or too heavy. Different types of needles will naturally

best suit different types of work; a heavier needle (Fig. 39C) will better penetrate the cellulose wall of a cell than a slender one. The needle tip must now be bent, preferably slightly less than a full right angle, so that two points can be brought into contact (Fig. 36). The nature of the bend must also suit the work at hand. For cutting, a greater slope is needed; and for entering plant cells horizontally, a double bend with a gradually rising tip is necessary (Fig. 40). Péterfi has added several other dissecting tools, such as a micropincer, microknife, and microspatula made from the scale of a butterfly wing (Fig. 39D).

With moist chamber and needles made, we can proceed to dissect. The material, which must be thin and reasonably

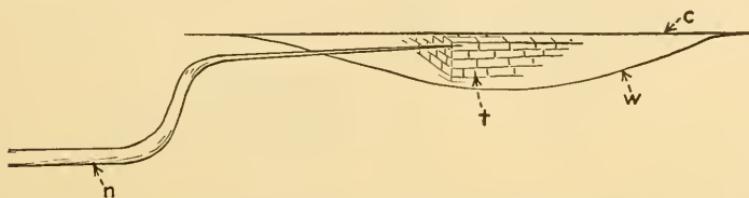


FIG. 40.—Plant tissue mounted in a hanging drop ready for dissection; *n*, needle; *t*, tissue; *w*, water film; *c*, glass cover slip.

transparent, may be an amoeba, an egg, the epidermis of a leaf, the plasmodium of a slime mold, etc. It is placed in a film of water on a glass slip. The latter is then inverted and placed upon the moist chamber. The two needle tips are brought into position under the living cell and gradually raised until the points are in the cell. The needles are then moved in any direction by the thumbscrews of the instrument.

J. Comandon and P. de Fonbrune have used another method to prevent evaporation of the water in which the material is suspended. They have injected a droplet of blood, containing the cells in which they are interested, between a drop of oil and the surface of the cover slip. The outer covering of oil prevents evaporation and yet permits the entrance and manipulation of microneedles and pipettes. The method eliminates the necessity of a moist chamber, thus permitting dissection with the droplet exposed without danger of drying out.

Microinjection.—The effect of salts on protoplasm is occupying the attention of cell physiologists at present more than is the anatomy of the cell. Tissues can be immersed in salts,

and much learned thereby, but such immersion experiments are always limited by the selective and protective effect of the protoplasmic membrane. If the salt is injected into the cell, a different reaction may result from that taking place when the cell is simply immersed in the solution. Injection is accomplished by micropipettes made just as are needles, except that tubing is used instead of rod. A needle first results; the tip is then broken off, leaving a tiny opening which, with practice, may be made as small as 2μ . The method of de Fonbrune permits making the pipette directly and of any desired size. The solution in the pipette is controlled by a plunger (syringe). For the isolation of bacteria and spores, the mouth of the investigator or a bulb gives sufficient control of pressure and suction, but for microinjection, a more precise method is needed. A

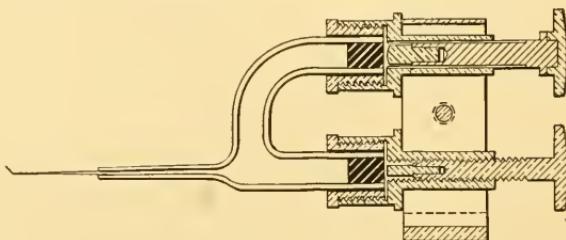


FIG. 41.—The mechanically controlled micropipette of Taylor.

hypodermic syringe, controlled by hand, is often used. Taylor has devised a screw-controlled pipette which is capable of great accuracy (Fig. 41). It is filled with mercury which is forced outward or drawn inward by screws. The liquid to be injected into a cell is first drawn into the tip of the pipette. The pipette is then made to pierce the cell, and the liquid forced into it. A little of the cell protoplasm may be withdrawn, or a cell part such as the nucleus may be removed by applying suction to a pipette the tip of which is within a cell.

A number of other methods for controlling micropipettes have been devised, some involving the thermal control of the expansion and contraction of mercury or air. The earlier models of heat-controlled pipettes did not operate with precision, but the newer one of de Fonbrune can be very accurately controlled.

Microelectrodes.—Attempts to measure the electrical potential of cells have led to many ingenious designs of microelectrodes.

The cell contains electrolytes which, being unequally distributed, must give rise to potential differences in the cell. It is such potentials which micrurgists have attempted to measure. There is also a potential between one cell and another in tissue and between the interior of a cell and its surrounding fluid. The results of such attempts we shall learn later. Here we are concerned with how they were made.

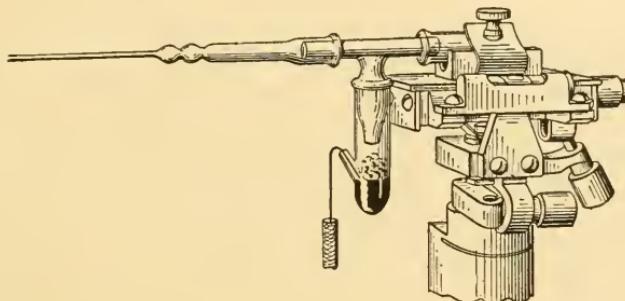


FIG. 42.—Agarpipette electrode with calomel cell of Ettisch and Péterfi.

Among the first microelectrodes was that of Ettisch and Péterfi. It consisted of a micropipette (fine capillary) filled with agar, or gelatin, saturated with a solution of potassium chloride which served as the conducting medium for the feeble electric current (Fig. 42). Electrical connection with an instrument (galvanometer) for detecting the presence of the current was made through a small calomel cell. Agar dissolved in an aqueous solution of an electrolyte and of such concentration

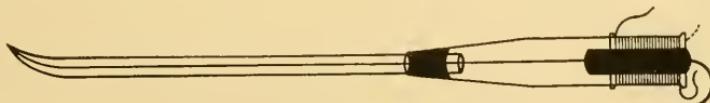


FIG. 43.—Micromagnet of Taylor.

(2 per cent) as to set to a gel is used instead of a wire to make contact within the cell, because metals bring about electrical disturbances which may give rise to potentials that are not normally present in the cell (as do zinc and copper strips when put into an electrolytic solution). Among the metals, platinum is the least objectionable. A neat addition to micrurgical tools is the micromagnet designed by Taylor. The figure

(Fig. 43) reveals the construction of the magnet better than words.

The ingenuity of these micrurgists seems to have no limit. Not satisfied with pulling cells apart, injecting salts into them, and determining their electrical potentials, they must measure their temperature. This has been made possible by Whitaker's microthermocouple. Thermocouples of larger size have been known for some time. They are instruments for measuring

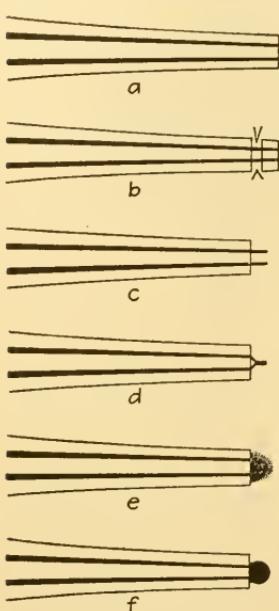


FIG. 44.—Microthermocouple of Whitaker, showing successive stages in its construction.

temperature electrically by means of a double electrode of two different metals. A thermocouple consists of a circuit of two different metals joined at two points to form junctions. If the junctions are of different temperatures, an electromotive force exists which is proportional to the temperature difference; it is, however, likewise dependent upon the metals used. Either a galvanometer to measure current or a potentiometer to measure potential thus also measures the temperature difference. The two metals must constitute a standardized combination, *e.g.*, iron against platinum, bismuth against an alloy of bismuth and tin, and iron against an alloy of gold and palladium. Such combinations indicate a certain degree of temperature when the electromotive force recorded is a certain microvoltage (thousandths of a volt). These two latter combinations give respectively 95 to 100

and 45 μ v. per degree of temperature (centigrade). Forty-five microvolts per degree means that 45 μ v. occur in the circuit when the temperature difference of the two junctions is 1°C. Ninety microvolts indicates 2°C., etc. The electromotive force per degree difference is just the same no matter how small the wires or junctions are. The device thus measures temperature difference, but this, of course, becomes temperature direct if the temperature of one junction is set or known; *e.g.*, one junction may be put in a constant-temperature bath or chamber containing a mixture of ice and water at 0°C., whereupon the difference

measured between the temperature of that junction and the other working junction gives the temperature of the second working junction (above or below 0°C.)

Owing to the great sensitiveness of electrical measuring instruments, temperature can be measured to exceedingly small fractions of a degree. It became Whitaker's task to make a thermocouple of microscopic size. He fused wires of different metals in glass or quartz (Fig. 44). By very delicate technique the two wires and the quartz are brought to a microscopic tip with the wires slightly protruding from the capillary. The wires are then joined by electroplating (Fig. 44*f*) to form a "sensitive junction."

Experimental.—The number and variety of experiments which have been performed with micrurgical instruments are many. The simplest among them, such as poking an amoeba with a needle point, reveals much of interest. The first thing to note is whether the amoeba "minds" it. Were he a dog or even a worm, he would give signs of distress if a sharp needle were pushed into him, but an amoeba is a droplet of protoplasm so small and relatively undifferentiated that one cannot be certain that he will "mind." It is, however, sufficient to know that an amoeba is protoplasm, a bit of living matter, to know that he will exhibit a "nervous" response to an external stimulus. An amoeba, when poked with a needle, either draws up into a ball, or, if a more bellicose fellow, he will "try" his best to get away. In saying that an amoeba tries, we bring up a very fundamental biological and philosophical question. When man does something, we believe that he "knows" what he is doing. When a dog does something, those of us who like dogs think that he, too, knows what he is about. When a worm does something, we are not quite sure that he knows; and as for an amoeba—a mere microscopic drop of protoplasm—most of us are quite certain that he does not know anything. But let us see if an amoeba is quite as stupid as he looks. If we push a needle into him, he may round up, or, as often, "run" away. This is not a mere fairy tale but an experimental fact. If an amoeba is "ambling" along and "hunting" for food, he will increase his speed if suddenly pierced with a needle and will move in a direction away from the needle (Fig. 45). The mechanist (believer in a purely physical interpretation of vital phenomena) will tell us that the

needle caused a pronounced decrease in surface tension at the point of entrance, leaving the anterior portion of the amoeba in a state of high surface tension which caused increased movement toward that end. This may be true; nevertheless, the result is that the amoeba quickly moves on, *away* from the needle. Whether we call it "intelligence," "nervous response," or "tropism," the simple fact remains than an amoeba will move away at an increased rate of locomotion from a disturbance which

is causing it "discomfort."

But our amoeba is even cleverer than this. When tightly held, he, on "realizing" that he is inextricably caught, pinches off that part of his body which is held by the microneedle and, leaving this bit of himself behind, hurriedly departs for other regions. Again, whether we romance about it or not, the experimental fact is there—the amoeba does the clever thing.

We shall leave for the philosopher to decide the difference between a fox chewing off one of his legs when caught in a trap and an amoeba pinching off part of his body when caught by a needle.

The reaction of an amoeba to mechanical irritation and the changes (*e.g.*, thixotropic collapse) which protoplasm undergoes on dissection indicate that micrurgical operations may cause very serious alterations in the behavior of an organism and in the properties of protoplasm. This is true, and critics who have never operated a microdissection needle like to tell about it. "Your cell isn't normal! How do you know that your results mean anything?" When a research worker in medicine studies the action of the heart of an animal under anesthesia, he is working with an abnormal organism. When exposed muscles and nerves are studied, they are under abnormal conditions. When animal tissues and plant seedlings are grown in culture solution, the environment is abnormal. When cells are treated with salts, when body fluids are withdrawn and then studied,

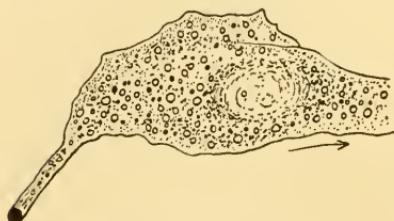


FIG. 45.—A living Amoeba pierced by a microneedle. The Amoeba is rapidly moving away from the needle which is "distressing" it. Part of the body of the microscopic animal has already disappeared from view on the right. The long protrusion of protoplasm stretched by the needle will be cut off by the Amoeba itself close to the main part of its body and left behind. (From a photograph.)

when living tissues are stained—in general, whenever chemicals are added or instruments applied to organisms and cells—the material is abnormal. In other words, any living object when subjected to an experiment is in a more or less abnormal state. This is experimentation, and on it does the advancement of biology depend.

The extent to which protoplasm will tolerate dissection is, at times, slight and, at other times, very great. Pieces may be repeatedly cut off from an amoeba, and the animal still lives.

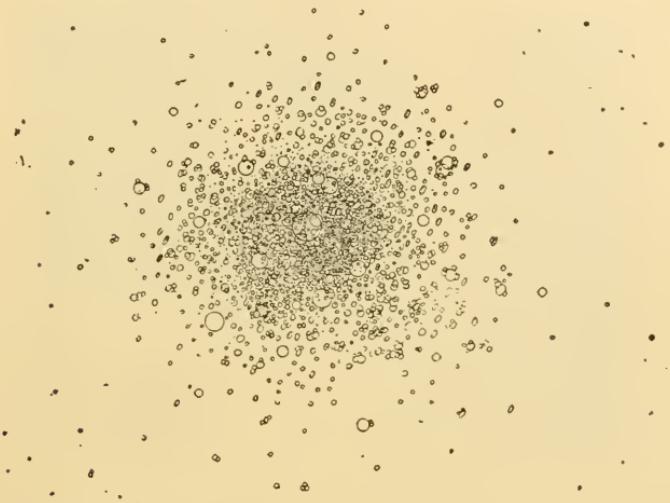


FIG. 46.—The rapid disintegration of a *Fucus* egg from mere puncturing with a needle.

Three amputations of a goodly portion of its body, until the amoeba was reduced to nearly one-fifth of its original size, left it, in one experiment, in a living condition. After the fourth operation, too small a remnant of its body remained to permit the amoeba to survive long; it gave up in despair, coagulated, and died! The amount of tolerance to injury depends, in part, on the way the operation is performed. Slow movement of the needles tends toward tolerance; rapid movement may cause immediate disorganization. Often a cell, the egg of a seaweed for example, is so sensitive to dissection that the mere prick of a needle will cause an instantaneous disintegration (Fig. 46). Protozoa sometimes explode in a similar manner at the slightest touch. On the other hand, protoplasm will often tolerate a

great amount of dissection with apparent indifference. The plasmodium of a slime mold may be badly torn, and the streaming of the protoplasm stopped, only to be resumed within a minute or less, around the wound.

Micrurgy has had to do primarily with the structure of cells and the physical properties of protoplasm, such as viscosity, elasticity, miscibility, and the nature of the surface membrane. These properties will be discussed in separate chapters. Here

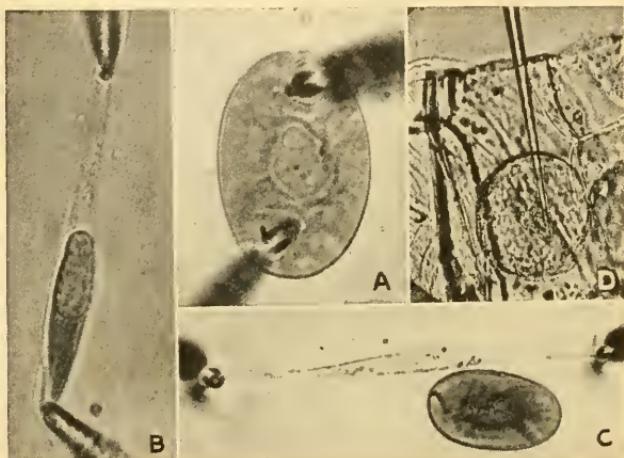


FIG. 47.—Stages in the dissection of a blood cell (*A, B, C*). In *B* the hemoglobin has escaped from the upper (pale) half of the cell (the nucleus is in the center), while it is still within the lower half of the corpuscle which has been stretched but little; *D*, a needle entering a living plant cell.

we can, for the moment, select any one of them and make it our task to solve it by micromanipulative methods. For example, has the red blood cell or the nucleus of *Amoeba* a morphological membrane? This question has been frequently asked. As material of blood cells we can best use the corpuscle of the amphibian *Amphiuma*, for they are very large. Two microdissection needles are brought up into the corpuscle (Fig. 47A) in such a way as to avoid the nucleus. The needles are then separated, and the cell first stretched (Fig. 47B) and then torn. It tears not like a mass of jelly but like a sac. The hemoglobin first escapes through the membrane where stretched and damaged most (Fig. 47B, upper half), then through the entire membrane (Fig. 47C). Finally, when the membrane is actually torn and opened, the nucleus escapes. An empty sac is left behind.

The Amoeba nucleus can be liberated by pinching off a bit of the animal with the nucleus. When thus freed and penetrated by two microneedles, the (now coagulated) nucleus is found to possess a very delicate membrane which can be lifted off like a thin veil (Fig. 48).

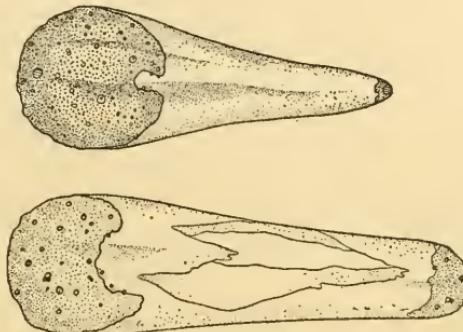


FIG. 48.—The isolated, degenerate Amoeba nucleus from which the coagulated nuclear membrane has been partially separated by stretching.

Plant tissue, in spite of its rigid wall, serves as interesting material for a variety of studies. Very satisfactory is the epidermis of onion scale leaves, when properly handled. These cells are alive, even though the onion has rested for weeks in storage. If a bit of the epidermis is peeled off and placed in a sugar or salt solution of suitable concentration, the living protoplasm in each cell shrinks up into a ball within the cellulose box that constitutes the cell casing. This is the phenomenon of *plasmolysis* (page 194). If a strip of this tissue is cut across with a sharp knife, some of the cells, *i.e.*, the rigid cellulose boxes, will be cut close to their ends, leaving the living protoplasm exposed and untouched within (Fig. 8). The epidermis is then mounted in a hanging drop of water on the underside of the cover of the moist chamber (Fig. 40). A specially constructed needle is used; it is manipulated so as to enter the cell through the open end and to come into contact with the naked protoplasm (Fig. 47D). The ball of protoplasm can be indented (Fig. 49), and an idea of the turgidity of its vacuole gained.

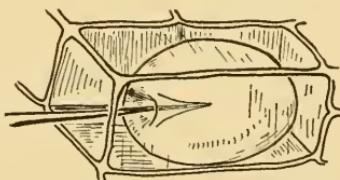


FIG. 49.—The exposed living protoplast of a cell indented by a needle.

The protoplast can be pulled out of the cell and dissected, when properties of the isolated nucleus and vacuole can be determined, such as the elasticity of the nucleoplasm, the nature of its membrane, tolerance to dissection, and the effect of reagents. Or we can direct our attention to the cellulose wall. The needle, if sharp and rigid, can be forced through several cells, penetrating the walls with ease (Fig. 8). Thus are some of the mechanical properties of cell walls in the natural state revealed.

The consistency of protoplasm has received much attention from investigators. One method of ascertaining it is to observe the behavior of protoplasm when needles are moved about in it. The method does not permit of great accuracy but has several advantages, such as permitting the various regions of a cell to be explored. A novel method of determining protoplasmic consistency, and its elastic qualities as well, is to insert in it a metal particle which is then attracted by an electromagnet (Fig. 35). The rate of movement of the particle gives an indication of the viscosity of the protoplasm, and the distance the particle returns when the magnetic force is removed is a measure of the elasticity. A more satisfactory method of ascertaining elastic values is to stretch protoplasm between microneedles. The living substance is ordinarily highly elastic. Convenient material for proving this is the protoplasm of slime molds or the exposed protoplasts of the epidermal cells of the onion just described. A needle is brought into contact with the protoplasm; the latter, being sticky, adheres to the needle and can then be stretched to great lengths (Fig. 111). The effect of salts on the extensibility of protoplasm can be ascertained by such experiments. It is found that the monovalent cations sodium, potassium, and lithium, decrease the stretching limit of protoplasm and that the bivalent metals cadmium and strontium increase it, while magnesium has no effect.

Microdissection studies may be carried out on the human corpuscle. The dissection of the human erythrocyte represents the ultimate in micrurgical technique. The blood cells measure but 8μ (0.008 mm.); the needle must, therefore, be the finest that it is possible to make and be manipulated with perfect control. It has been possible to tear open the human red corpuscle and to establish the presence of a delicate membrane, as in the case of the much larger amphibian corpuscle.

C. V. Taylor of California has taught us much about the behavior of the protozoan *Euplotes* by micromanipulative methods. He was able to sever various parts and thus learn their function. By cutting all of the neurofibrils (Fig. 50), or selected ones among them, with quartz microscalpels, he could observe the effects of the cutting and subsequent regeneration upon the previously analyzed stereotype behavior of the organism. But the most dramatic of micrurgical operations done by Taylor was the isolation of the micronucleus, by means of which he discovered its function. *Euplotes* (Fig. 28) has two nuclei, a large worm-shaped one, the macronucleus; and a very small globular one, the micronucleus. Taylor neatly extracted the micronucleus with a micropipette and observed the subsequent behavior of his patient. Fifty-four *Euplotes* were thus operated upon, and none lived more than five days. The absence of the micronucleus is therefore not immediately fatal, but without it *Euplotes* can neither eat nor divide (reproduce). The organism lives only as long as the food within it lasts. A justified criticism would be that the operation itself and not the absence of the small nucleus was the cause of the inability to eat. That this is not true was shown by extracting some of the cytoplasm elsewhere in the cell, *i.e.*, by performing an operation similar to the first but leaving the nucleus untouched, and further, by removing parts of the large nucleus from 22 individuals, each of which continued to live and form thriving colonies. But the final and most convincing proof that the loss of the micronucleus and not the operation was the cause of the abnormal behavior was had when Taylor extracted the micronucleus from each of two *Euplotes* and immediately replaced them. Both organisms gave rise to thriving colonies.

George Scarth has done valuable micrurgical work on plant material, to which we can best refer in subsequent chapters. Freundlich and Hauser have applied micrurgical technique to the study of the structure of the latex globule in rubber solutions. These globules, which are present in all latex-producing plants

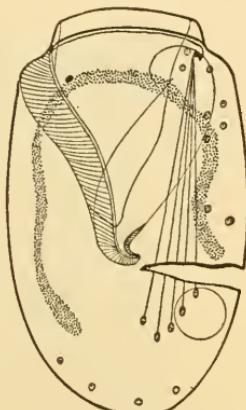


FIG. 50.—The neurofibrils of *Euplotes* cut by a microneedle. (From C. V. Taylor.)

(Hevea, Fig. 89; Euphorbia; milkweed; etc.), contain the chief hydrocarbon constituent of rubber. They are about the size of the human red blood corpuscle. To tear them apart with delicate needles gives some idea of their physical structure.

Microinjection is one of the most promising fields of investigation in micrurgy. Its possibilities have only just been entered upon. The effects of salts and dyes are different when injected into protoplasm from what they are when the cell is bathed in them. Microinjection has shown what these differences are.

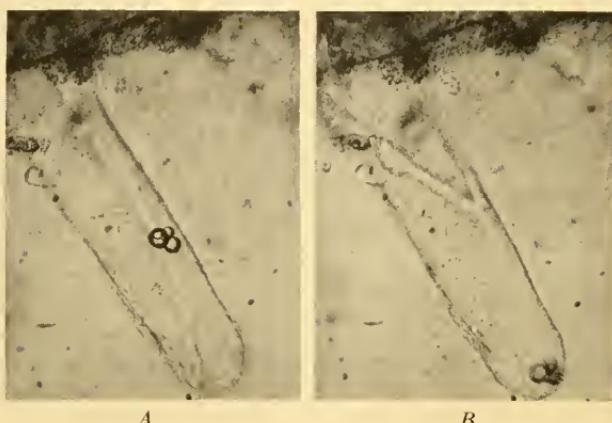


FIG. 51.—Root hairs of *Limnobium* with oil globules injected. At the upper left in A (just over what appears to be a C) is seen the coagulum which has healed the wound where the micropipette entered. The glass-like strands of protoplasm (best seen in B) are streaming. (From T. Kerr.)

“Indicator” dyes have been injected into protoplasm, and by color changes the acidity determined. Needham of England has performed such experiments and ascertained not only the acidity but the oxidation-reduction potential of a cell. Janet Plowe injected into cells dyes which do not penetrate when the cell is immersed in them. It was her problem to ascertain if the inability of a dye to enter a cell is due to properties of the protoplasm as a whole or only to peculiar properties of the surface layer. She found the latter to be the case, for the dye diffuses freely in the protoplasm when injected directly into it. Kerr has injected a variety of substances into root hairs of the aquatic plant *Limnobium*. If oil droplets are injected (Fig. 51A), they may be seen to travel down to the end of the cell (Fig. 51B), carried by the streaming of the protoplasm.

Many are the possibilities of micrurgy.

CHAPTER V

TISSUE CULTURE

The cell is the ultimate seat of all vital processes, but these processes do not depend on the activities of one cell alone, for the multicellular organism is a united, harmonious whole. This means that happenings in higher organisms are the expression of the correlated behavior of many cells, each dependent upon the other and upon their environment. These truths are the working hypothesis and the philosophy of the "new cytology." The old cytology dealt primarily with the individual cell considered apart from its environment. The new cytology has to do with the relationship between the living cell and its neighbors near and far and the fluids which bathe them all.

The study of dead (fixed and stained) material has revealed much of value. The series of events involved in cell division—indeed, the very existence of the chromosomes and many similar details in cell anatomy—were first made known through the study of killed tissues. But such studies, important as they are, have had to do mostly with the form and structure of cells, often neglecting function. Structure is of significance only in so far as it explains function.

The German physiologist Schwann, one of the authors of the cell theory (1839), called attention to the importance of body fluids in the life of the cell, but cytologists continued to center their attention on anatomy, until the French physiologist Claude Bernard (1878) again pointed to the necessity of considering the medium in which cells are bathed. One of the modern advocates of this point of view is the French-American experimental surgeon Alexis Carrel, who has repeatedly emphasized that cells are in physiological continuity with their environment and that only with their environment do they constitute a whole.

The living cells of tissues can rarely be studied within the body and therefore within their natural environment. The cell physiologist was, therefore, confronted with the task of dupli-

eating this environment so that cells could be grown and studied outside the animal body in comparable surroundings. Attempts to accomplish this were made many times and in a number of ways. The first efforts involved the isolation of entire organs from the body. These were simply kept alive in solutions, no growth taking place. The English physiologist Ringer found (in 1880) that the heart of the frog when perfused with a salt solution (of sodium, potassium, and calcium chlorides) would continue to beat for some time after its removal from the body. The German Ludwig artificially circulated a fluid through the blood vessels of an excised organ and thus obtained the survival of glands for a number of hours. Modern methods have revived this older technique in a much improved form. With the aid of a carefully controlled pump, a respiratory chamber, and aseptic conditions, parts of the body perfused with a nutrient fluid can be kept alive outside the body for several weeks. But such methods, though of great value to general physiology, are too gross to permit the study of individual cells.

Knowledge of the living cell has been greatly augmented by studies based on the technique known as *tissue culture*, which is the cultivation of cells and tissues *in vitro* (literally "in glass" but meaning "outside the organism," as distinguished from culture *in vivo*, *i.e.*, "within the body"). Tissues can be isolated from the body and grown in culture media in such a way as to permit microscopic observation of individual cells under the highest powers of the microscope during growth under controlled conditions. Two biologists, the German botanist Haberlandt and the American zoologist Harrison, independently made the first experiments which led to the culturing of isolated tissues. Haberlandt was the first to attempt to grow isolated cells and tissues in culture, and Harrison the first to succeed in doing so. Haberlandt was unsuccessful primarily because he used plant material, but he pointed the way, as his words show:

To my knowledge there have as yet been made no organized experiments to cultivate isolated vegetative cells of higher plants in a suitable medium; and yet the results of such culture experiments would throw some light on those qualities and potentialities which the cell as an elementary organism possesses. They should also reveal something of the changing relations and mutual influences to which the cells, within the many-celled organism as a whole, are exposed.

Harrison succeeded in doing with animal material what Haberlandt predicted should be possible and thus paved the way for all of the remarkable work in tissue culture which has since been done by Carrel and Lewis in America, Strangeways in England, Fisher in Denmark, Levi in Italy, and others.

Harrison was interested in the development of the nervous system. He realized that there was need for a method in which the behavior of certain cells could be observed when removed from the bewildering conditions existing within the body. The ordinary methods of histology were inadequate to answer the question of the origin of the nerve fiber. Here was a problem that demanded for its solution some new form of technique, a method that would permit the growth and study of isolated nerve cells. Consequently, in 1907, Harrison placed small portions of the various tissues of the frog embryo in drops of lymph suspended over the depression in a hollow-ground glass slide. The lymph coagulated and formed a substratum, and the cells grew. During the days that followed, Harrison observed the formation of protoplasmic threads from the cells and was thereby able to show that embryonic nerve cells form long processes which can be identified with nerve fibers in the embryo. The fiber was seen to develop from the spinning of a thread of protoplasm by means of the amoeboid activity of the free end of a cell process.

Others before Harrison, in addition to Haberlandt, had the idea of isolating tissues and studying their behavior. Among these were Wilhelm Roux and Leo Loeb. The latter planted pieces of epithelium from the guinea pig in small blocks of clotted blood or agar, which were then placed for incubation in the body of another animal and subsequently studied by means of microscopic sections. But this procedure is very different from that of tissue culture as at present practiced and can hardly be said to have suggested it, although the underlying purpose was the same.

The experiment which Haberlandt attempted and Harrison brought to fulfillment Carrel perfected. Simply put, the technique consists in isolating a small bit of tissue, either from an animal embryo or from the actively growing tissues of an adult body, and placing this bit in a suitable medium. The preparation is then hermetically sealed, placed in an incubator at body temperature, and allowed to grow. The tissues most frequently

used are those from the chick embryo. The culture medium is one prepared to meet the particular requirements of the tissue. The receptacle should permit microscopic study. Either a depression (hollow-ground) slide or a Carrel flask will serve



FIG. 52.—Culture chambers; right, hollow-ground slide; left, a Carrel flask.

(Fig. 52). Suitable tissue is to be had in the chick embryo, preferably eight to ten days of age (Fig. 53), though any age and other animal embryos and adults serve well. The nature of the experiment determines the kind of tissue to be used. A typical experiment would proceed as follows: A five- to ten-day chick embryo is removed from the shell (Fig. 54) and placed in



FIG. 53.—A seven-day chick embryo.

a watch glass containing some saline solution. The chick heart is isolated and cut into numerous tiny pieces. One of these bits is placed either in a Carrel flask or on a cover slip in a suitable amount of culture medium (Fig. 55). If a cover slip is used, it is then inverted and sealed on a depression slide (Fig. 52) or placed on a vaseline ring made on an ordinary slide (Fig. 55). The flask or slide is then placed in a constant-temperature oven at 37.5°C. and allowed to incubate. Within several hours or a day,

cells will have migrated out from the tissue, forming a fringe of very loosely joined cells around the so-called *explant* (Fig. 56). If the explant is a bit of heart tissue, it may, even though it is but a twentieth part of the original organ, continue beating for many days after isolation. It is one indication, of which there are many, that the culture is alive and normal. More extraordinary is the observation of W. H. Lewis of a single cell of the chick heart in culture which pulsated with the same rhythm of

the entire heart. The rhythmic contraction of muscle fibers (*e.g.*, skeletal muscle) may also be observed in culture.



FIG. 54.—Removing a chick embryo from its shell.

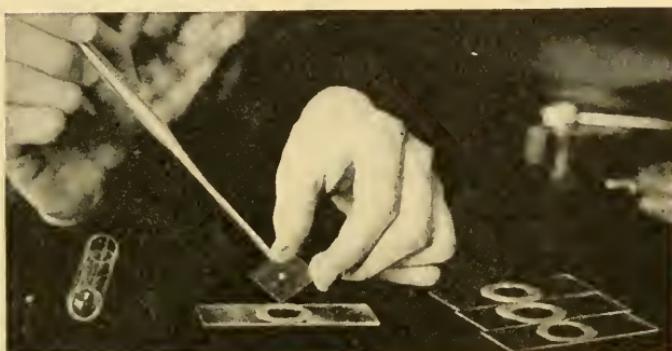


FIG. 55.—Mounting a bit of chick tissue on a cover slip (the culture chambers in this case are made by forming a ring of thick petroleum jelly on an ordinary slide).



FIG. 56.—Cells (fibroblasts) migrating from the parent tissue (explant) of chick heart. (From a fixed and stained preparation.)

The most fundamental of the various problems of the technique of growing tissue cells in culture is that of nutrition. But

before knowledge of this had progressed much beyond the pure salt-solution stage, other important problems were partially solved, notably that of a suitable substratum for the cells to grow upon. Cells must have a solid surface to cling to if active growth is to take place. The best substratum so far used is blood plasma (plasma is the medium in which the corpuscles of blood are suspended). Agar, gelatin, glass wool, silk gauze, spider webs, cotton fibers, etc., have all been tried, but while any one of them can be used as a framework upon which the cells may grow, none gives so uniform a growth as does the fibrin in plasma. Lymph was first used (lymph is a body fluid and coagulates like blood), but later Burrows substituted the plasma of blood. It is obtained by centrifuging whole blood; the upper layer is plasma. The liquid plasma is put in a Carrel flask. Coagulation quickly follows. On this coagulum the tissue is placed and covered with nutrient solution.

Still another condition necessary to keep tissues alive for a great length of time is washing. The flask must be opened, or the slides unsealed, the tissues thoroughly washed (with the nutrient or a saline solution), a fresh supply of the culture medium added, and the respective receptacles resealed. Carrel describes his discovery of the need of washing as follows: At the beginning, the cultivation of tissues consisted in the brief survival of a fragment of fresh tissue in a drop of body lymph or blood plasma; but after a few days, the cell degenerated and died, owing to waste products, lack of food, or both. Washing in Ringer solution every two or three days proved remarkably successful in prolonging the duration of life. Tissues which are to grow for months or years must be regularly and frequently washed, yet when neglected they may show surprising vitality. A bit of chick heart, sealed in a hollow-ground slide, immersed in culture medium and left untouched for 18 days may still exhibit active beating. Washing, however, prolongs the time. Carrel tells of a fragment of heart which continued to pulsate 104 days after its extirpation from a chick embryo. Cells migrated, multiplied, and remained normal. But the total mass of the tissue did not increase. A suitable substratum and frequent washing are not sufficient. Nutrition, other than a pure salt solution, is necessary.

The question of a suitable nutrient medium for the cultivation of animal tissue and of plants has a long history, beginning some

fifty years ago, when Ringer found that a frog's heart removed from the body would beat longer if suspended in a saline solution and still longer if a small amount of calcium chloride were added. On the basis of these experiments, Ringer made the solution which now bears his name. The ingredients and proportions are as follows:

NaCl.....	9.0	grams
KCl.....	0.42	gram
CaCl ₂	0.25	gram
H ₂ O.....	1,000	cc.

Ringer's solution was modified for one purpose and another before the days of tissue culture in the strict sense. Ringer himself had tried the addition of other salts. From such experiments Locke developed a solution which has been much used in the cultivation of cells and tissues:

NaCl.....	9.0	grams
CaCl ₂	0.24	gram
KCl.....	0.42	gram
NaHCO ₃	0.2	gram
H ₂ O.....	1,000	cc.

A similar solution is that of Tyrode of the following composition, now used in tissue culture:

NaCl.....	8.0	grams
KCl.....	0.2	gram
CaCl ₂	0.2	gram
MgCl ₂	0.2	gram
NaHCl ₃	1.0	gram
NaH ₂ PO ₄	0.5	gram
Glucose.....	1.0	gram
H ₂ O.....	1,000	cc.

Fragments of the heart, posterior limb, or any other part of the chick embryo will usually survive in such solutions for a week or more at a temperature of 37.5°C. Pure salt solutions, such as the first two listed, will, if slightly modified (*e.g.*, Pfeffer's solution), serve a normal whole plant quite well, because plants have the ability to manufacture organic food from inorganic material. Animals lack this capacity. Consequently, while cells from body tissues will live for a time in a Ringer solution or even in a simple saline (single-salt) solution, yet cells do not live long, nor

do they grow, *i.e.*, increase in number; cell division rarely occurs. Cells live in pure salt solutions only as long as the residual energy (stored reserves) with which they were endowed when they were placed in these solutions lasts. When the food reserves have been used, no further growth can possibly take place in the absence of utilizable proteins. Distinction must, therefore, be made, in the case of animal cells, between a nutritive medium (containing protein or protein derivatives) and a "protective" medium such as Ringer's solution or sea water. In drawing such a distinction, one must remember that it is always exceedingly difficult to decide in the last analysis between what is nutritive and what protective. It cannot be arbitrarily stated that all inorganic elements are protective and all organic ones nutritive or that known nutritive compounds are not also protective. Yet it is true that in certain (inorganic) solutions animal cells live for a time but do not grow. We may regard these as protective. It becomes necessary, therefore, to find a culture medium that will promote growth. Again Carrel had a happy thought. He added to the cultures a drop of embryonic juice, the fluid taken from crushed embryos and therefore the same kind of fluid in which the tissue is bathed when it is growing in its normal place in the developing organism. The result was most successful. We shall let Carrel tell about it in his own words:

A truly wonderful effect was immediately observed. Fibroblasts began to multiply about the tiny pulsating heart muscle, which was soon surrounded by a large amount of tissue in which it disappeared. The tissue went on growing and could be divided into two parts, which also grew rapidly. Every forty-eight hours, the cultures were washed in Ringer solution by Ebeling or myself, divided into two parts, and cultivated again in embryonic juice. Today, hundreds of experiments are made every month with the pure strain of fibroblasts descended from the tiny fragment of pulsating tissue that I possessed in 1912.

A strain of cells (fibroblasts) derived from this bit of embryonic heart muscle started by Carrel on Jan. 17, 1912, is growing today, twenty-three years later (Fig. 57). The rate of growth is tremendous. The number of generations is now over 4,000. Each cell divides, on an average, twice in forty-eight hours, thus doubling the size of the colony.

The fact which impresses one most forcibly and which is of the deepest biological significance is the sustained vitality of the cells. Today, after twenty-three years of growth and possibly over 4,000 divisions, proliferation is as active as it was at the beginning. The rate of growth is undiminished, unaffected by time. Life is immortal, but only in a sense, which, when we analyze it, becomes commonplace. In the case of the tissue cultures it is immortal only under the favorable conditions existing in carefully controlled cultures. The life of cells in tissue culture is immortal only because there is survival but not of the original cell. Its progeny have survived, and this is true

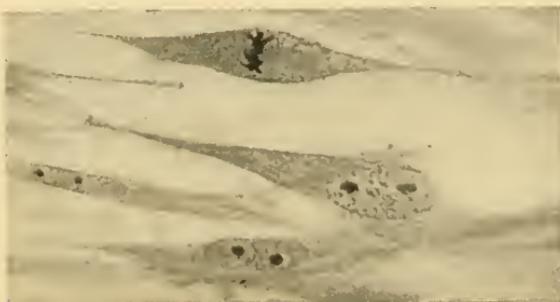


FIG. 57.—Cells from the original "old strain" of Carrel started in 1912 (upper cell dividing with chromosomes at center). At this time the culture was thirteen years old (stained preparation): $\times 1000$.

throughout nature. The life of man is immortal in that it survives through its progeny. Weismann enunciated this fundamental principle early in modern experimental biology when he stated that the Protozoa are immortal. The technique of tissue culture has produced nothing new from this point of view, but it has made it possible to keep a strain of somatic (body) cells alive in a manner not heretofore accomplished.

The embryonic chick cells cared for by Carrel and his associates have, through their progeny, grown for twenty-three years. It must, therefore, be granted that any and every normal living cell possesses these same immortal potentialities, but it is quite evident that a special environment is necessary, an environment which embryonic cells possess but which becomes altered with the attaining of mature age. What has the adult animal acquired or lost which causes the cells of the body to age, to lose the vitality of youth? Through tissue culture we have one answer to this

question. Toxic secretions are formed which stop growth. Unless these are removed, aging results. We grow old because we poison ourselves. If the requisite environment is produced, an old cell may regain its youth; its latent potentialities for unlimited growth are revived. This fact plays an important part in life, in those extraordinary cases of rejuvenation of parts in lower organisms, in wound healing, and in tumor growths. In each of these instances, old cells carrying on their usual functions, suddenly begin to grow. They are stimulated into active growth when adjoining tissue is wounded, as, for example, in the loss of the claw of a crab or the tissue otherwise irritated. The



FIG. 58.—Living culture of chick heart showing migration of cells (taken with dark-field).

remaining cells become young again and produce a new part, heal a wound, or form a tumor. It seems, therefore, that cells are young or old or, let us say, behave as though young or old, not because of time but because of environment, of body fluids. Poisonous waste products produce age. Freedom from these and fresh solutions in their place give youth.

Yet another important feature of the technique of tissue culturing remains to be referred to. It is subculturing. Where continued and active growth over a long period of time is desired, subculturing must be done. This involves the making of new cultures, by dividing the old one and transplanting a bit of it on a new substratum. This is possible only when the cultures are grown on coagulated organic material such as blood plasma. In this manner has Carrel kept his cultures going many years, though cultures may live months by simply washing and adding fresh nutrient solution without subculturing.

It was formerly thought that blood plasma was relatively devoid of nutrition for cells, that serum (plasma is serum plus fibrin) even when obtained from a very young animal, not only fails to supply fibroblasts with the nitrogen necessary for multiplication but actually inhibits their proliferative activity and shortens their life *in vitro*. But more recent work has shown—

what might have been expected of any product of blood mechanically separated—that plasma is nutrient in itself though not sufficient for very active growth.

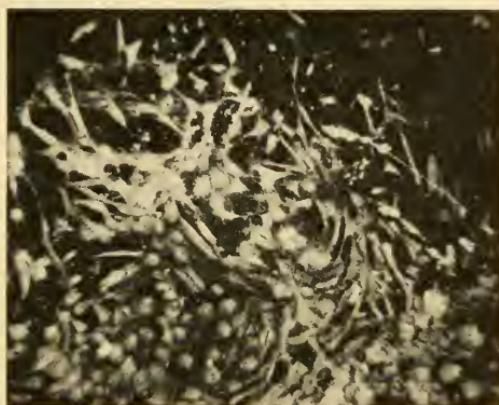


FIG. 59.—Part of living culture of fibroblasts (dark-field).

The manner of growth of tissues in culture is strikingly different from that in the body. After a short recovery period of several hours, cells in culture begin to wander out from the explant (Figs. 58, 59). It is interesting that cells which in the animal body cling together to form tissues should in culture separate and wander away from each other, maintaining only a

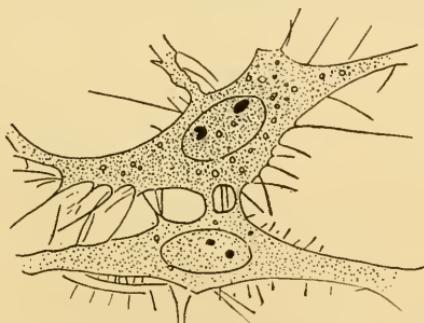


FIG. 60.—Fibroblasts in culture showing intercellular connections (stained preparation).

slight contact, just as if they were independent organisms, which indeed in a sense they do become. The cells crawl by a process comparable to amoeboid movement. At first, there is merely a migration of cells. Later, multiplication by cell

division (mitosis) takes place, with chromosomes clearly visible (Fig. 57). It is believed that in certain types of cells (fibroblasts), division and multiplication do not take place if the cells become fully isolated from each other. A single isolated cell in culture does not multiply. Delicate intercellular connections are usually visible and often very numerous (Fig. 60).



FIG. 61.—Drawing of human liver tissue in culture (culture supplied by Dr. J. P. M. Vogelaar and drawn by Miss Ann Stiles).

The types of tissues which have been grown in culture are many. Practically all parts of the chick embryo—nerve, epidermis, bone marrow, etc.—grow well. Cells of bone marrow from adult organisms migrate very rapidly. Within an hour after explantation they have spread over a large area. Other tissues which have so far been cultured are the skin of the frog, white blood corpuscles, tissues of rat, guinea pig, man, etc. Human tissue has been successfully grown, though not extensively so. Ebeling was one of the first (in 1914) to cultivate normal and sarcomatous human tissue. Since then, several other workers have experimented with similar cultures; thus, Castrén succeeded in keeping strains of fibroblasts obtained from a

human being growing for over half a year. A recent successful growing of human tissue in culture is that of Vogelaar. He has grown human liver and thyroid glands from a three months' embryo (Fig. 61).

Cells in culture are most often studied as living material, yet they may be killed and stained, and further observations of value made upon them (Figs. 56, 63).

The types of cells which are to be found in a culture (*e.g.*, of a chick embryo heart) are numerous. The best known and most striking are the fibroblasts (Figs. 59, 62). It is these which Carrel has had growing for twenty-three years. They are among

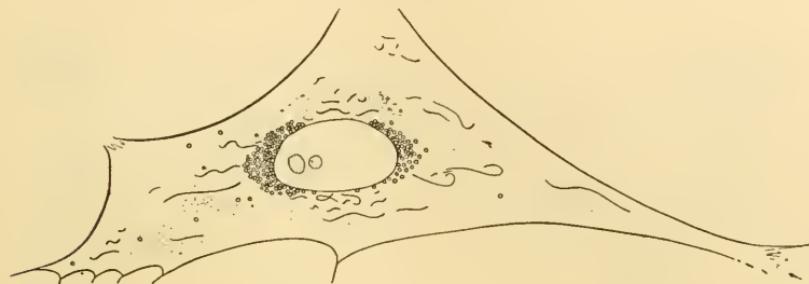


FIG. 62.—Drawing (semidiagrammatic) of a fibroblast.

the largest of tissue cells, distinguishable by size, mode of colony formation, nature of surface, cytoplasmic elements, appearance of nucleus, angular shape, and long protoplasmic processes formed as they move. Almost as abundant are the monocytes and macrophages, types of blood leucocytes. They are less angular, more active than fibroblasts, have undulating membranes, a very different locomotion, and are independent. Other types of cells occurring in culture are muscle, nerve, and epithelium.

The appearance of a cell may be greatly changed by its inclusions, which, in addition to the nucleus, include five main types (in cells from the chick-embryo heart), *viz.*, vacuoles, neutral-red vesicles, neutral-red granules, fat globules, and mitochondria (Fig. 63). The number and distribution of these cause quite different appearances in the cells. The granule-like inclusions are often massed close to the end of the nucleus (Fig. 62). Such a region Carrel and Ebeling have termed the "digestive area." A starved cell shows no such aggregation of food and waste products.

It is not always an easy task to distinguish the types of cells which appear in culture. The difficulty is due to the fact that embryonic cells are less differentiated than are mature ones. Growth in culture tends toward dedifferentiation, while growth in the body tends toward differentiation. The extent of the dedifferentiation in culture is in part dependent upon the nature of the culture medium. Certain types of cells become transformed into other types. This tendency toward transformation is known as *polymorphism* and occurs also normally within the body but is accentuated in culture.

Polymorphism, or the capacity to exist in many forms, is very characteristic of tissue-culture cells. It is a property that one

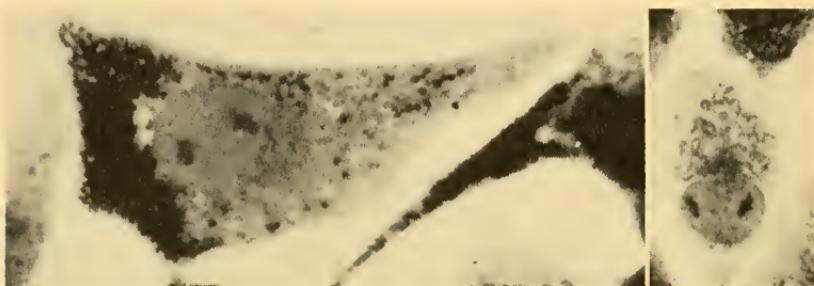


FIG. 63.—Fibroblasts showing (left) aggregation of fat globules; and (right) worm-shaped mitochondria.

might well expect cells to have when they are embryonic, for their destiny is then a vague and distant thing. Whether they are to become nerve cells, blood leucocytes, fibroblasts, or macrophages is not yet fixed, or, if partly predetermined, development in that direction has not progressed so far but that their fate may still be altered. Those two factors which primarily determine the destiny of cells are origin and environment; the latter appears to predominate. The problem is one of wide occurrence in nature. In the eastern tropics, the pitcher plant *Nepenthes* forms low rosettes with many pitchers when growing on wet, boggy ground. On dry, solid ground, the plant grows as a long vine and forms no pitchers. The difference may be due simply to moisture, though salts are probably a determining factor. A single tropical species of the genus *Vaccinium* may, in the same locality, occur as a tree, shrub, or vine and be terrestrial or epiphytic (*i.e.*, on the soil or off the soil on other plants). The causes of this polymorphism are unknown.

While the character of the medium is apparently the chief factor causing structural variation in tissue-culture cells, others play their part. Monocytes, like blood leucocytes of which they are a variety, are scavenger cells. After filling themselves with degenerate cells and other waste matter, they become many times larger and are then known as macrophages. The macrophages are grown-up or, shall we say, filled-up monocytes. They may take in as many as 50 cells of their own size. Such transformations have been found by W. H. Lewis to be of very common occurrence in cultures of blood from a variety of animals. It is not, perhaps, quite correct to say that gorging brings about the transition of monocytes into macrophages but rather that "giant" cells arise from either of the two, which are different forms of a very plastic basic type of cell. Monocytes and macrophages may also arise from and become transformed into fibroblasts. Carrel and Ebeling found that fibroblasts, treated with plasma and heparin, may become macrophages. They acquire all the physiological properties of macrophages and remain indefinitely in that state. The macrophage and the fibroblast are therefore apparently functional variations of the same basic cell type.

This seeming lack of stability in type of cells in tissue culture is offset by a strong tendency of cells to remain true to type, at times. Ebeling and Fischer have shown that strains of epithelium and fibroblasts cultivated side by side in the same medium keep definite individual characteristics even though they may change in external form. Thus, certain cells which are morphologically (structurally) indistinguishable from fibroblasts retain, after many months in culture, original physiological properties not possessed by fibroblasts. Vogelaar has grown embryonic human liver which, though in culture (Fig. 61), shows a pronounced tendency to produce a network and form typical trabeculae which are structural features characteristic of the adult liver. Many of the cells possess two nuclei, another property typical of liver (epithelial) cells.

Heredity and environment determine the destiny of living organisms. By heredity is meant that physical and chemical constitution that the cell acquires when it is first formed. Environment involves many factors, from temperature, moisture, and salts to the presence of other cells and the position of a cell in

the organism. The influence of these last environmental factors is illustrated by the classical experiment which involves the growth of a single cell, or *blastomere*, separated from a young frog embryo. It has been found that if the cells of a 2-celled frog embryo are separated from each other, each will develop into a normal frog. This is also true even of the 4-, the 8-, and the 16-celled stage; that is to say, each of the 2, 4, 8, or 16 cells of an embryo is potentially capable of producing a whole frog. Each of these cells, before separation from the others, was destined to become a part of a frog, but now, after separation, their destiny is changed, and each cell gives rise to all the tissues of an entire frog. (In actual experiments, not all cells grow when separated; it is necessary that only one of the embryonic group should grow to prove our point.)

The problem of the destiny of a cell arises wherever many types of tissues have a common origin, which is rather generally true throughout nature. A single initial cell in the growing point of certain roots and stems (Fig. 20) gives rise to all the diverse tissues of the mature root or stem. It may be said that cell type is determined by function, but how does the cell when young "know" what its function is to be, so that it may develop into the kind of cell which it must become in order to fulfill that function? The most satisfactory solution of the problem is that which places the responsibility for the function and destiny of the cell upon its relative position in the organism. Any other cell would, if taken young, carry on the same function if it found itself in the same position. In tissue cultures, relative position has no meaning because there is little differentiation, and that little is determined by origin, nutrition, and time.

Another peculiarity of tissue-culture cells is their occasional multinuclear condition. The rule among cells is one nucleus apiece (exceptions have been cited). In culture, it is not uncommon to find cells which have six or even a dozen nuclei. H. D. Fell, W. H. Lewis, and others have described how this multi-nuclear condition may arise. Sketches by Fell and Andrews (Fig. 64) show how a binucleate cell results from incomplete division. At 3:07 o'clock, the chromosomes are on the equatorial plate; *i.e.*, division is half complete; at 3:10, the chromosomes have reached their respective poles; at 3:20, nuclear division is complete, and cell division well underway; at 4:07, the two

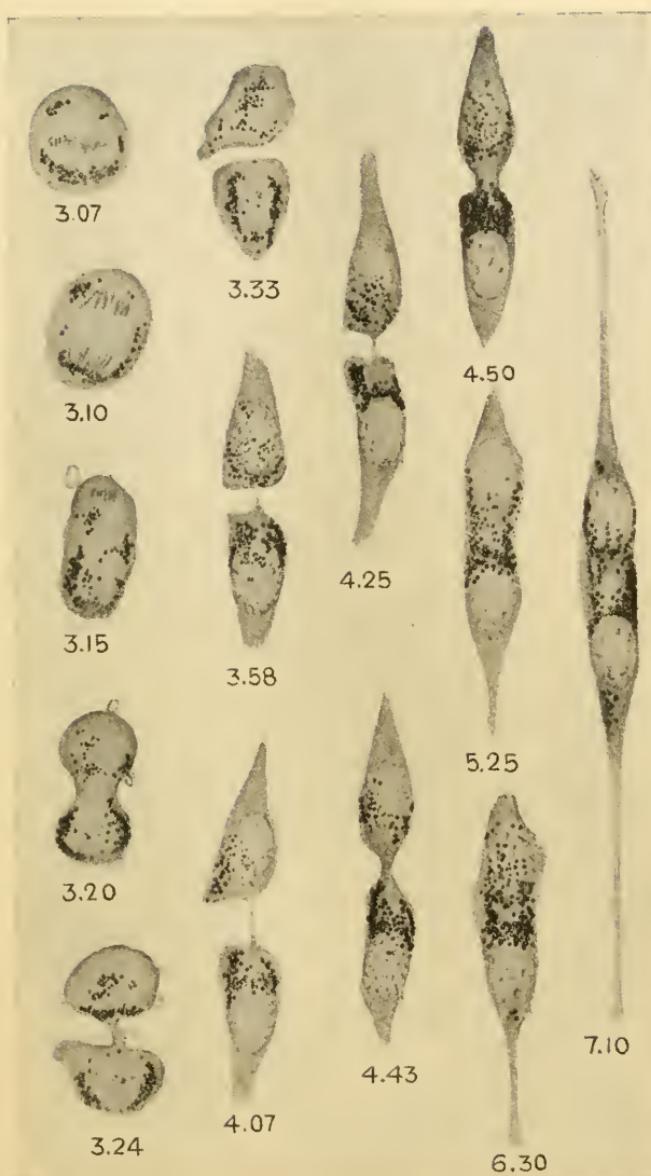


FIG. 64.—Stages in division resulting in a binucleate cell. (From H. D. Fell.)

daughter cells are nearly separated, but a protoplasmic thread still connects them; at 4:43, the two cells, instead of fully separating, as they normally should, come together again; and at 5:25, one single cell remains with both of the daughter nuclei.

Of great significance to the whole field of physiology is the problem of the chemical nature of growth-stimulating substances. Important work dealing with this problem has been done by Carrel and his coworkers. Lewis showed that cells will live, migrate, and divide in a pure salt (sodium chloride) solution. They do better in a "balanced" salt solution and apparently still better in a solution containing sugar (glucose), but their most prolific multiplication occurs when an organic substance of greater chemical complexity is present. Carrel discovered that cells would live indefinitely in embryonic tissue juice. He, Ebeling, and Baker set out to ascertain the chemical nature of the substances in embryo extract that are responsible for continued growth. One would suspect these to be protein in nature, and this was found to be true. Substituting amino acids was not successful, even though these hydration products of proteins serve well as substitutes in the nutrition of animals. The proteoses, which are more complex products of the proteins, proved to be suitable for sustained tissue growth. It seems, therefore, that the extraordinary growth-producing powers of embryo extract are due to certain peptic proteins. While the proteoses apparently play the chief role, the complete diet must also contain nucleic acid (an important constituent of chromosomes), glycocoll (the simplest of the amino acids), glutathione (a dipeptide, concerned in the respiration of cells), and oxyhemoglobin. The addition of each of these, successively, to an artificial culture medium, in which no embryo juice is used, increases the rate of growth. Embryo juice either contains all of these, or they can be made by the growing cells from the proteins of the embryo juice.

Much of the work on nutrition has been on sarcomatous (cancer) fibroblasts. It appears that the requirement of cancer cells differs slightly from that of normal ones. Normal cells use artificially supplied proteins only for a short period of growth, while sarcomatous fibroblasts multiply for a long time in them.

Vogelaar has developed a nutrient medium with Ringer solution, phosphate, glucose, and peptone as the base, to which

he has added insulin, hemin, cysteine, and thyroxin. These last three substances were chosen because each of them contains an important element; hemin contains iron; cysteine, sulphur; and thyroxin, iodine. Each of these substances is important for growth.

Again and again we find reason to regard protein as the fundamental substance which underlies life. Salts, carbohydrates, and fats will keep the living machine going, but to make new protoplasm a protein base is needed.

Every problem in cell physiology would be elucidated, at least to a degree, if subjected to study by the technique of tissue culture. Several such problems have been mentioned. In addition, there are such purely physical ones as the viscous, elastic, and glutinous state of protoplasm and the mechanism underlying chromosome migration. Then there are physiological (if not also philosophical) problems, such as the nature of death. Strangeways, in order to illustrate to his students that there are two types of death, that of the body as a whole and of the individual cell, was wont to purchase fresh sausage at the market and from its ground meat make a number of cultures of which one or two always formed thriving colonies of cells. In the Carrel laboratories, growth in culture has been obtained from fragments of tissue kept for five or six days in cold storage.

Of great importance is the problem of the healing of wounds. If Carrel could, from studies on the influence of substances on the rate of growth of cells in culture, hasten the rate of reparation of tissue ten times, a cutaneous wound would heal in less than twenty-four hours, and a fracture of the leg would be mended in four or five days. Experiments by Lecomte du Noüy have shown that the rate of cicatrization (healing) of a sterile wound is a function not only of the area of the wound but also of the age of the patient; that is to say, the area of the wound being the same, the rate of repair is faster in young individuals than in old. Later work by Carrel, Ebeling, and Baker demonstrated that these findings may be explained on the basis of progressive chemical changes which take place in the blood plasma during the lifetime of the individual, in other words, age. Parker demonstrated a marked difference in the action of infant and adult serums on colonies of fibroblasts from subcutaneous human tissue. One-half of them were treated with

serum obtained from a fourteen-months'-old infant, and the other half with serum from a twenty-seven-year-old adult. The cultures treated with the infant's serum attained an area in ten days which was over 150 per cent greater than that reached by those treated with adult serum.

Finally, there is the ever present and tragic story of cancer. Fischer has placed cultures of cancerous and normal tissues side by side and watched how the cells of the former penetrate the framework of the latter. Carrel, Baker, and Ebeling have found that glycocoll increases the rate of growth of sarcomatous (cancer) cells about 70 per cent. If it is glycocoll that causes unlimited growth of cancer cells in our bodies, perhaps the growth could be checked by control of excessive glycocoll production.

There is one possible criticism of tissue culture as a method of cancer study. Cells growing in a drop of solution on a glass slide in an incubator are in an environment which, although in many respects like that in the body, is nevertheless an artificial one. There is no blood stream, no nervous system, no coordinated body. Will the culture cells, therefore, remain identical with others of their kind within the organism? In certain respects they do, but many differences exist, and one of the most significant of these is that tissue cells, like cancer cells, grow on indefinitely. Normal body cells grow until a mature organ is formed and then stop. Only abnormal cells, such as those of a tumor, grow on and on. They lack a "purpose" in life. Such lawless growth is also characteristic of cells in tissue culture. This leads one to suspect that cells in culture possess a type of abnormality like that of tumor cells. The persistence in type shown by some cells in culture is evidence against this criticism; cells in tissue culture do adhere to certain heritable tendencies and do not become wholly abnormal.

Some interesting and important bits of evidence support the foregoing statement that cells in culture retain certain of their original characteristics over a long period of time; thus, cancer cells in culture will produce cancer in an organism inoculated with them some months after the culture is started, while normal cells in culture never acquire this property.

Culture methods also throw light on the much discussed problem of the possible morphological distinction between normal and cancerous cells. It is maintained by some that while can-

cerous tissue is, as a whole, well differentiated from normal tissue of the type from which it arose, it is not possible to recognize an individual cancer cell under the microscope. A general disarrangement of the tissue characterizes the cancerous region, not the individual cells. On the other hand, certain differences have, so it is believed, been found; thus, the malignant cell is generally coarser and more refringent than the normal one. Peculiarities of the chromosomes of cancer cells have been described. In culture, W. H. Lewis finds that all the malignant cells studied are certainly cytologically and culturally different from normal cells, with a possible exception of the spontaneous adenocarcinomas of the mouse. The various rat sarcomas and carcinomas are not only different from normal but different from one another. So far, says Lewis, no two strains of malignant cells have been found to be exactly alike. Several different strains of cells cultivated for from one to four years have retained their malignancy and their cytological and cultural characteristics.

M. J. Hogue has used tissue culture as a means of studying the effects of drugs on cells. Baumgartner and Payne have developed a technique for the study of insect reproductive cells in which certain abnormalities seen in culture do not appear because the body pressure and specific ferments are not disturbed. The technique consists in anesthetizing a male grasshopper and attaching it to a glass slide by means of melted paraffin which is run around the insect so as to form a small chamber in which the grasshopper and salt solution rest (Fig. 65). The preparation may be maintained for several hours. Before the grasshopper recovers from the effects of anesthesia, the testes are drawn out through an aperture in the abdominal wall. The connective tissue, which encloses the tightly packed follicles, is torn away with a needle, and the follicles float out into the surrounding medium. They remain attached, at the proximal end, to the vasa efferentia and may thus be studied while still connected to the living animal.

G. C. Hirsch has studied mouse pancreas while it is still attached to the animal. The mouse is narcoticized, and its pancreas so

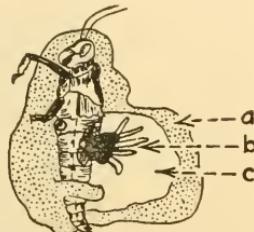


FIG. 65.—Tissue from a living anesthetized grasshopper dissected out into a solution: *a*, paraffin wall, *b*, follicles, *c*, nutrient solution. (From Baumgartner and Payne.)

isolated as to leave it attached to the body by a strand through which blood flows. With the tissue in Ringer's solution, Hirsch studied the cellular activity for a period of thirty hours, particularly in reference to secretion and cytological changes as the result of treatment (with pilocarpin, etc.).

Still another method for studying tissue is that developed by the anatomist E. R. Clark. The lobe of a rabbit's ear is perforated, making a hole about an inch in diameter which is covered with two thin plates, one of glass and one of mica, held very close together. During the process of healing, the new tissue grows between the plates, forming a very thin layer so that the growing cells and blood vessels can be seen with great clarity as through a window.

Plants.—Haberlandt was mentioned in the introduction of this chapter as the originator of the thought that isolated cells would grow in culture. He was a botanist and naturally tried to prove his idea by growing plant cells in a nutrient solution, but he failed. The culturing of plant cells should be, and every one at first expected that it would be, much easier than the growing of animal cells. Plants, in nature, show a very ready tendency to save themselves from destruction by developing new shoots and forming new individuals under most unfavorable conditions. A tree trimmed until only the trunk remains may burst forth with new shoots at the next spring. A fence post set when green may sprout. Cuttings of many plants grow very readily. The life plant *Bryophyllum* has the extraordinary capacity to develop a new plant from each notch in a leaf when the leaf falls to the ground. Many tropical ferns proliferate in this manner. Mere cutting of the veins of a *Begonia* leaf will cause a new plant to arise at each cut. Why do such cells, which exhibit rejuvenating powers far exceeding those in animals, not grow in tissue culture? In a sense they do, but not as undifferentiated tissue. They always form fully developed organs. It is as if the plant cell were determined to fulfill its purpose in life and form an entire plant or not grow at all!

A near approach to plant tissue culture is the growth of extirpated root tips. Such work has been done by Robbins, Kotte, White, and Gautheret. If the tip (1 cm.) of a young root from a sprouting seed is cut off and put in a Pfeffer salt solution with organic material, it will grow for weeks or months under favorable

conditions (Fig. 66). After the root is quite long, 1 cm. of its tip may be snipped off and put in a fresh solution where it continues to grow as before. Side rootlets are formed just as in the natural condition. But always only rootlets, never more than this and never undifferentiated tissue. P. R. White has kept the embryonic tissues of young roots actively growing for 32 months (and they are still growing at the time of this writing) but again always rootlets are formed and never fully developed roots. Tissue culture generally implies the growing of cells which show

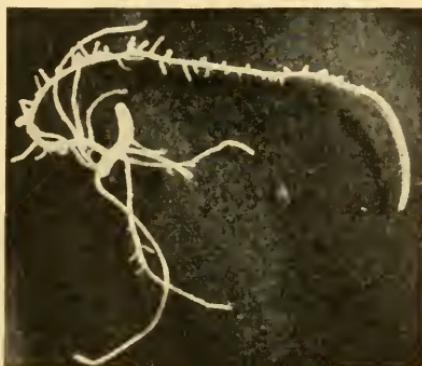


FIG. 66.—A detached 16-cm. root which has, in seventeen days, developed in culture from an extirpated 1-cm. root tip.

little or no differentiation, but this is not always necessarily the case; thus, kidney and pancreas keep to type. White regards his plant cultures as much tissue cultures as are those of kidney and pancreas which show differentiation, because fully developed organs, *i.e.*, mature roots, are never formed (secondary growth, *e.g.*, of xylem, is not produced).

There is another type of plant culture which has a resemblance to tissue culture, namely, that of myxomycetes, the slime molds. Only recently has it been possible to grow this primitive form of life continuously in the laboratory. It is grown on agar containing oatmeal. The culturing of slime molds is not very different from the growing of any fungus, but as it can be grown vegetatively without interruption much like tissue cells in culture, it is worthy of mention here, if for no other reason than that it, even more than animal tissue cultures, offers an abundance of undifferentiated protoplasm for physiological study (Fig. 1).

CHAPTER VI

THE COLLOIDAL STATE

Protoplasm consists essentially of three types of systems—a true solution (molecular dispersion) of salts, carbohydrates, and other water-soluble substances; an emulsion of fats and like matter; and a dispersion of organic substances, mostly proteins, which form jellies. The last two types of systems are *colloidal*. While it is impossible to attribute more or less importance to any one necessary substance or system in protoplasm, yet it is true that those substances which are colloidal, such as the fats and the proteins, possess properties which particularly characterize living matter. This is especially true of the proteins and the colloidal systems (jellies) that they form. It is thus obvious that a knowledge of the colloidal state of matter becomes exceedingly important to the study of protoplasm.

Characterization of the Colloidal State.—Matter is said to be in the *colloidal state* when it is permanently dispersed and so finely so that the individual particles, though larger than molecules, cannot be seen. The water of the Mississippi River is forever muddy because the clay particles contained in it are so small that they do not readily settle until they meet the salts of the sea, when they quickly fall and form the Mississippi delta. Both the suspension of the finely divided clay particles in the river water and their precipitation by the salts of the sea are colloidal phenomena. A threatening cloud is made up of droplets of water finely dispersed and in (relatively) permanent suspension in the air; the water is in the colloidal state. When the droplets, through coalescence, become too large, they fall as rain. The tails of comets consist of particles so small that when our earth sweeps through them we see nothing of them, yet illuminated against the black background of the night sky they become brilliant. The cosmic particles of the comet's tail are in the colloidal state, and their luminosity is due to the scattering of light, a colloidal phenomenon. The blue color of

tobacco smoke or pale forest-fire smoke, of mist, blue eyes, feathers, and skimmed milk is due to the presence of tiny particles in permanent suspension, in other words, to matter in the colloidal state. Metals may be so finely dispersed in water as to remain in permanent suspension. Gold so dispersed forms a classical colloidal suspension. Where dispersed particles settle, as does sand in water, or rise, as does cream in milk, the system is a coarse suspension. Only the smaller particles which remain behind in permanent suspension are colloidal. Minuteness in size of particles and (relative) permanency in suspension characterize the colloidal state.

The medium in which the particles of a colloidal system are scattered is termed the *dispersion medium*, or *continuous phase*; and the scattered particles are the *dispersed*, or *discontinuous phase*; thus, the air of clouds is the dispersion medium, and the droplets of water are the dispersed phase.

Matter finely divided and in permanent suspension is said to be colloidally dispersed rather than in solution, because the particles are above the molecule in size, though one may speak of colloidal solutions; furthermore, a molecular dispersion may be colloidal if the molecules are exceedingly large (as in the case of proteins).

As particle size is characteristic of the colloidal state, the latter may be (somewhat arbitrarily) defined in terms of the former. The maximum size of colloidal particles is conveniently placed at the limit of microscopic visibility. The minimum size is above that of the (average) molecule. This means that the largest colloidal particles are below $0.1\ \mu$ or 0.0001 mm. in diameter and therefore invisible and above $1\ m\mu$ or 0.000001 mm. The lower limit is often placed at $10\ m\mu$, though Zsigmondy claims to have detected colloidal gold particles as small as $3\ m\mu$.

This is the world of colloidal dimensions, the world which Findlay has picturesquely called "the twilight zone of matter."

Dialysis.—Colloid chemistry is said to have had its beginning in 1861 with the discovery by the English chemist Thomas Graham that certain substances in solution will pass through a parchment-paper membrane while others will not. Those that pass through Graham called *crystalloids*, because crystalline substances, such as salt and sugar, are typical of them. Those that will not pass through he called *colloids* (Gr. *kolla*, glue;

eidos, semblance), because substances such as glue are typical of them.

No science has its beginning with the work of one man, any more than has its subsequent development. It is claimed that the French chemist Baudrimont studied colloids seventeen years before Graham. But Graham gave us names for the substances and their behavior, and names cling.

Inability to pass through a parchment-paper membrane was, then, Graham's test for the colloidal state. To put a substance to such a test is to *dialyze* it; and the membrane, with its accouterments, is the *dialyzer*. The dialyzer which Graham used was

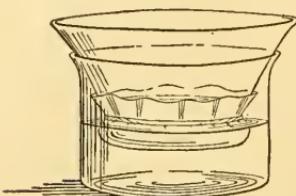


FIG. 67.—Graham's dialyzer.

a very simple affair (Fig. 67), parchment paper tied over one end of a large-mouthed funnel. The colloid or crystalloid is on one side of the membrane, and pure water on the other. Membranes for dialysis may be of a number of kinds. All animal membranes, such as a pig's bladder, serve well. Artificial collodion (celloidin) membranes are much used. These may be cast in the form of sacks into which the solution to be dialyzed is put; the sack is then corked and suspended in water. All these membranes have one property in common—they permit crystalloids (salts, etc.) to pass through but not colloids (proteins etc.). Through their use, colloidal substances may be freed of impurities of noncolloidal nature; thus, if it is desired to free blood serum of salts, dialyzing for many days, with repeated changes of water, will do it, as far as is experimentally possible. The salts leave by diffusion, if their concentration within the dialyzer is greater than that without. The process may be hastened by *electrodialysis*.

The simplest form of *electrodialyzer* is one in which one electrode is placed within a parchment or collodion sack, and its mate placed outside in the surrounding water. The electric field will hasten the outward diffusion of those ions of a sign opposite to that of the outside electrode. By reversing the current every few minutes and changing the water, both cations and anions will be alternately attracted by the outer pole. More efficient is the electrodialyzer of Pauli (Fig. 68). The apparatus consists of a central chamber, into which is put the substance to be

dialyzed, and two end chambers containing water. The latter contain the metal electrodes and are separated from the central chamber by parchment membranes. (A platinum electrode is used at the anode, as acid is formed here.) Current is applied, the water changed frequently, and dialysis continued until the desired purity is reached. In this manner Pauli has dialyzed the serum of horse blood for seven weeks and obtained what is probably the purest seralbumin yet produced.

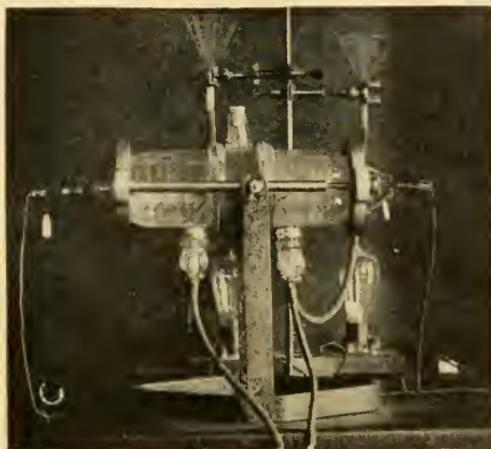


FIG. 68.—Electrodialyzer of Pauli.

The Two Major Groups of Colloids.—“A dominating quality of colloids is the tendency of their particles to adhere”; thus did Graham characterize the “gluelike” substances. But there is nothing gluelike about such fine suspensions as water droplets in air (clouds), carbon in air (smoke), clay and gold in water, and similar systems which are colloidal because they fulfill other prerequisites of the colloidal state, *viz.*, fine dispersion and permanent suspension. The question therefore arises whether the gluelike properties of such colloids as gelatin, albumin, and rubber or the minuteness in size and permanency of suspension of particles in such systems as clouds, smoke, and the like shall be the criterion of the colloidal state. If failure to pass through a parchment-paper membrane, *i.e.*, to dialyze, is the test, then both types of systems, the gluelike ones and the suspensions, are colloids. The French chemist Duclaux has attempted to restrict the term colloid to the gluelike substances, but colloidal

suspensions of solid particles are in other respects so typically colloidal that to exclude them from the colloidal state would be arbitrary and make for confusion. Yet we must agree with Duclaux in so far as to recognize two main and rather distinct groups of colloids, the suspensions and the gluelike substances. Among the characteristics distinguishing these two groups of colloids is the capacity of the gluelike substances to form *gels*, or *jellies*. (Certain of the metal (oxide) suspensions form gels and thus constitute a connecting link between the two groups.)

Objection to the term "colloid" has been voiced. The word is unfortunate, as are all words ending in *-oid*. To say that a substance is "like" something else is to admit ignorance of it except in certain particulars. But this is, after all, the way that all descriptions begin. The classical chemist disapproves of grouping substances so diverse as are gelatin (a protein), cellulose (a carbohydrate), charcoal (carbon), silica (sand), and colloidal gold (a metal) all under the one term colloid. But the classical chemist thinks in terms of chemistry, while the colloidal chemist thinks in terms of physics. All of the above enumerated substances, when in the colloidal (finely dispersed) state, have certain physical properties in common—they do not pass through a parchment-paper membrane, and they scatter light. Colloidal chemistry does not have to do with different *kinds* of matter but with matter in a characteristic state. Gold is still a metal, benzene a hydrocarbon, and albumin a protein when in the colloidal state. Colloids are different kinds of substances occurring in a similar and unique condition. It might, therefore, be well to avoid the word colloid and to speak instead of the "colloidal state" or of "colloidal systems." As such systems stand apart from others owing to their physical rather than their chemical properties, the subject dealing with them could better be termed "colloidal physics" rather than "colloidal chemistry."

Methods of Preparation.—Colloidal suspensions may be made by one of two general methods—dispersion or aggregation. Large masses may be dispersed or broken up into minute particles, or molecules may be brought together into aggregates of colloidal size. There are a number of ways of accomplishing both of these processes. Coarse matter may be dispersed mechanically, electrically, or chemically. A solid substance may be ground into a very fine powder which is shaken in water. Some of the par-

ticles will remain suspended. The method is a possible one but not usually very successful. It works best with glass, which if very finely powdered by grinding in a mortar will form a permanent colloidal suspension. When an oil is mechanically shaken in water under suitable conditions, a stable emulsion results.

Electrical dispersion is the most satisfactory method for making colloidal suspensions of metals. The apparatus was devised by Bredig and modified by Burton. A heavy wire of the desired kind (copper wire does well, as it is very pure and not costly) is fastened to the clapper of an electric bell. A second piece of the same wire is clamped near the first in such a way that the free points of the two are about an eighth of an inch apart and immersed in water. The wires are connected to a 110-volt house current with four or six lamps (two or three amperes) in the circuit (Fig. 69). The bell clapper is set going; its vibration keeps the two wires continually making and breaking contact so as to maintain an electric arc. After being in operation some twenty or thirty minutes, a suspension of the metal is formed due either to the disintegrating effect of the electric arc on the metal electrodes or to the formation of a gas of the metal, by the heat of the arc, which condenses into particles of colloidal size. The heavier particles settle, and the finer invisible ones remain in suspension, giving the water a translucent appearance of characteristic color. If the metal electrodes are of gold, a red, violet, or blue dispersion is obtained.

Chemical methods of preparing colloidal dispersions are numerous and varied. They are termed *peptization* methods. To *peptize* is to disperse. If the term is used in its broadest meaning it includes mechanical and electrical methods, but it is better to limit the expression to methods involving the addition of a peptizing agent. (The word is derived from "peptone," itself a peptizing agent.) In a sense, water peptizes gelatin, glue, gums, etc., in that it disperses them until a colloidal solution results. The dispersal of clay in water through the addition of ammonium hydroxide or through leaching with acid, the emulsi-

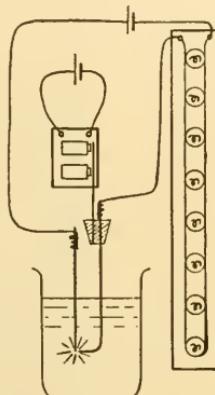
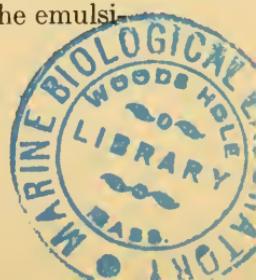


FIG. 69.—The Bredig-Burton method of electrical dispersion of metallic colloids.



fication of dirt (from cloth) with the aid of peptizing agents such as soap, proteins, and gums, and the emulsification of oil in water with the aid of soap and proteins are all dispersion methods by peptization.

The second general method of preparing colloidal solutions involves the aggregation of dispersed molecules. (For this Graham introduced the term *pectization*; it is, however, not much used.) Such *condensation* methods include the replacement of the solvent (as when a few drops of an alcohol solution of gum are dispersed in a large quantity of water); reduction of a metallic salt to its metal ($2\text{AgOH} + \text{H}_2 \rightarrow 2\text{Ag} + 2\text{H}_2\text{O}$); oxidation ($2\text{H}_2\text{S} + \text{O}_2 = 2\text{S} + 2\text{H}_2\text{O}$) decomposition; ($\text{As}_2\text{O}_3 + 3\text{H}_2\text{S} = \text{As}_2\text{S}_3 + 3\text{H}_2\text{O}$); and hydrolysis ($\text{FeCl}_3 + 3\text{H}_2\text{O} = \text{Fe}(\text{OH})_3 + 3\text{HCl}$). All involve the aggregation of molecules of soluble substances into colloidal particles of insoluble substances. The preparation of colloidal iron oxide (or hydroxide) is readily carried out. Several small pieces of ferric chloride are dropped into boiling water. The salt is immediately converted into the oxide or the hydroxide, which is insoluble in water and therefore remains suspended in colloidal form. A rich brick-red dispersion results. If oxygen is passed through an aqueous solution of hydrogen sulphide, colloidal sulphur results. (The oxygen of the air is usually sufficient.) Stannic chloride often reacts similarly; a solution of it may become cloudy, the salt having hydrolyzed instead of ionizing.

Tyndall Effect.—The optical properties of colloidal systems particularly distinguish them from molecular dispersions. Among these properties, two are very characteristic—the Faraday-Tyndall effect, commonly known as the Tyndall cone; and structural color. The first of these ties all the diverse types of colloidal systems together—the solid and liquid suspensions and the gluelike colloids. All show a Tyndall cone. The Tyndall phenomenon may be illustrated by a commonplace event, which, however, is not truly colloidal. When a ray of sunlight creeps through the crack of a shutter into a darkened room, the particles of dust suspended in the air, which are not visible when the room is well lighted, become bright spots of light because they are illuminated laterally against the dark background of the room. If, now, we perform a similar experiment but with truly colloidal "dust," by allowing tobacco smoke

to enter a beam of light in a partially darkened room, we see the smoke brilliantly illuminated. If, instead of smoke particles in the air, metal particles in water are illuminated laterally against a dark background, an even more striking cone of light is obtained (Fig. 70). It was such a cone of light that Faraday first saw, and Tyndall later interpreted.

A dispersion of silver oxide prepared by reduction of a silver salt with dextrin gives a particularly interesting Tyndall cone, in that the cone is green where the light beam enters, then yellow-green, yellow, yellow-orange, orange-red, and brilliant red at the far end. This is probably due to color absorption by the solution.



FIG. 70.—The Tyndall cone (in a silver solution).

There are a number of other phenomena similar to the Tyndall effect which are not to be confused with it, *viz.*, luminescence, opalescence, iridescence, fluorescence, and the Raman effect. Luminescence is a purely chemical (oxidation) process. Opalescence and iridescence are due to colloidal structure but of plates, or lamellae, and not particles. Fluorescence is unpolarized light coming from certain substances (sodium salicylate, quinine sulphate in water, mercury vapor) when illuminated, the light given off being of a different (greater) wave length than that illuminating the substance. (The light from colloidal dispersions shows characteristic polarization.) A number of light effects result in a change of wave length. This is true of fluorescence, the Compton effect (scattered homogeneous X rays which give rise to a change of wave length), and the Raman effect. (The last will be referred to again presently.)

The Ultramicroscope.—That colloidal systems consist of minute particles in permanent suspension was early suspected.

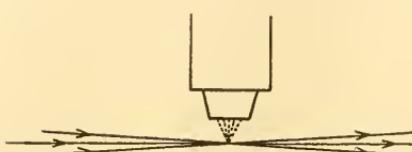


FIG. 71.—Lateral, *i.e.*, indirect (dark-field) illumination.

Faraday suggested this when he first showed a cone of light in colloidal gold to the Royal Society. Proof of the presence of such particles is to be had in *ultramicroscopic*, or *dark-field*, illumination. The principle of the ultramicroscope is the same as that of the Tyndall cone.

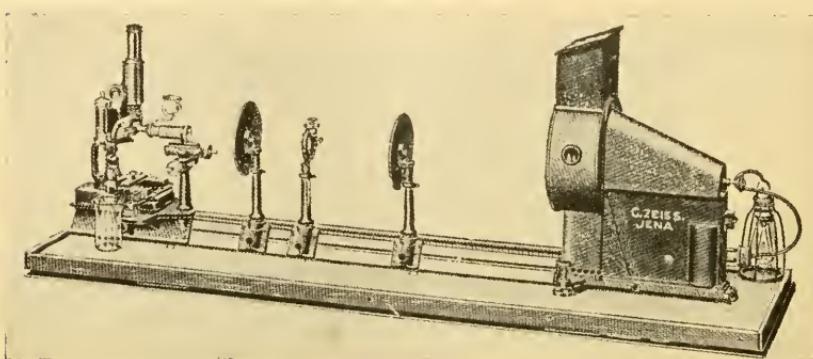


FIG. 72.—The ultramicroscope. (Carl Zeiss.)

In both cases, colloidal matter is illuminated laterally against a black background, with the result that microscopically invisible particles become "visible" because of the light that they scatter. The direct beam of light from the source of illumination does not enter the eye of the observer; only the scattered rays from the particles are seen (Fig. 71). The colloidal particles thus illuminated appear as brilliant spots against a black background. Two main types of lens systems are used to give lateral illumination—the cumbersome equipment known as the *ultramicroscope* (Fig. 72) and the simple *cardioid condenser*, or *dark-field illuminator* (Fig. 73).



FIG. 73.—The cardioid condenser. (Arthur H. Thomas.)

The development of the ultramicroscope is due to the Germans Siedentopf and Zsigmondy. The instrument designed by them is a large and expensive affair which others have attempted to simplify. This type of ultramicroscope is sometimes referred to as the slit microscope, the slit being a small aperture corresponding to the hole in the shutter of a darkened room into which a beam of light enters and illuminates the motes in the air. The ultramicroscope consists further of a series of lenses and apertures, apart from the microscope proper, which direct a light ray into the colloidal material and thus illuminate it laterally. The Tyndall cone of a colloidal solution, as seen with the naked eye, is the total effect of the scattering of light by many particles, no individual particles being visible. In the ultramicroscope, the individual particles are "seen" as centers of a burst of light which surrounds each one of them. Thus viewed, a colloidal solution resembles the Milky Way at night but with every "star" dancing about in active Brownian movement.

The Siedentopf-Zsigmondy ultramicroscope has, because of its inadaptability, given way to the much simpler and more convenient dark-field condenser. It was invented by F. H. Wenham in 1850. The same fundamental principles underlie this instrument as those of the slit type of ultramicroscope, *viz.*, a black background and indirect illumination. The light, however, enters the condenser from below, as in an ordinary microscope. This is possible because the rays strike, and are reflected by, two successive mirrored surfaces which direct and concentrate them at a point in the colloidal solution (Fig. 73). The illuminating beam of light which passes beyond this point does not enter the microscope; only the scattered rays from the colloidal particles are visible.

There are numerous types and modifications of the dark-field condenser, such as the *cardioid*, the *paraboloid*, and the *change-over* condensers. The first two names indicate the nature of the curve of the reflecting surface. The change-over condenser permits going from direct, as in the ordinary microscope, to dark-field illumination, without changing condensers.

A simple form of dark-field illumination is the *central stop diaphragm* (Fig. 74). It is used successfully with an Abbe condenser. The central stop excludes all rays within the field of

the objective's aperture. The beam of light used for illumination must be larger than the central stop.



FIG. 74.—Central stop diaphragm.

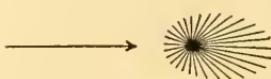


FIG. 75.—The unsymmetrical scattering of light by colloidal particles.

An ingenious development of dark-field illumination is the *Spierer lens*. It was designed by Charles Spierer and is based

on the principle that a colloidal particle scatters light unevenly in such a way that there are more rays given off in one direction than in another, *viz.*, in the direction of the illuminating ray (Fig. 75). Obviously, then, the visibility of ultramicroscopic particles depends upon the angle of the illuminating ray. If a colloidal particle is viewed toward the source of illumination, the observer will then see the maximum amount of scattered light. A brighter picture, smaller particles, and a finer structure will consequently be discernible. The principle of the Spierer lens is simple; with its accouterments it is illustrated in Fig. 76. Observation toward the source of illumination, with at the same time dark-field, are accomplished by placing a tiny metal mirror of silver, gold, platinum, or aluminum (*m*, Fig. 76), within an oil-immersion objective. This metallic reflector covers but a small part of the lens surface. Light comes up directly from below through a small aperture, passes through and illuminates the colloidal matter, and enters the lens. Here it strikes the

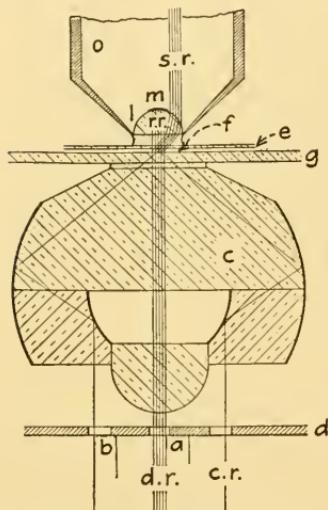


FIG. 76.—The Spierer lens and special (Zeiss-Spierer) cardioid condenser: *o* = microscope objective; *l* = lower lens of the oil-immersion system; *m* = platinum (Spierer) mirror; *r.r.* = reflected rays from the (Spierer) mirror, *e* = cover slip; *g* = slide; *c* = cardioid condenser; *d* = special fixed diaphragm; *a* = 1.5-mm. aperture for direct light, *d.r.*, to Spierer lens; *b* = slit for cardioid rays, *c.r.*

below through a small aperture, passes through and illuminates the colloidal matter, and enters the lens. Here it strikes the

mirror from which it is reflected downward again, thus illuminating the particles a second time from above. The first illumination is, however, the important one, for it directs the maximum brilliancy of the ellipse of scattered light into the lens. The presence of a mirror in the lens prevents direct light from passing beyond the objective to the eye of the observer, but it does not hinder scattered light from the particles entering the lens around the mirror. In order that only as much direct light shall enter as can be reflected by the mirror, the aperture of the iris diaphragm below the microscope stage must be at least as small as the mirror.

The Spierer lens is a dark-field system in itself; it may, therefore, be used with an ordinary Abbe condenser if the diaphragm is closed to a pinhole. But it is more convenient to use it with a special cardioid condenser (*c*, Fig. 76) which has a fixed aperture (*d*) of correct size (as small as the mirror). The use of a cardioid dark-field condenser with the Spierer lens adds still another ellipse of scattered light. The Spierer optical system has brought to light an interesting structure in cellulose (page 256).

All dark-field methods of illumination depend upon diffraction phenomena for their usefulness. This is both an advantage and a disadvantage. The diffraction of light by ultramicroscopic structure makes that structure evident, but the picture revealed may not be an exact duplicate of the actual structure. It may be merely a diffraction image. This is, however, not necessarily true, but one must be cautious in interpreting dark-field pictures. The great advantage lies in the fact that if interference in any form is obtained, then colloidal structure is certain to be present, and usually the dark-field picture tells the type, even if it does not always give an exact counterpart of the structure.

Structural features in protoplasm that are invisible and others that are but faintly visible with direct light are brought out vividly with the aid of dark-field illumination. Such illumination also greatly facilitates the observation of bacteria.

Orders of Magnitude and Particle Size.—In order better to grasp the dimensions that characterize the colloidal state, it will be well to stop a moment and recall the ultramicroscopic scale. A *micron* is one-millionth of a meter, or one-thousandth of a millimeter, and has the symbol μ . This unit does for microscopic objects; thus, a human blood corpuscle or an average globule of

butterfat in milk is 8μ across, and a bacterium is between 1 and 5μ long. Ultramicroscopic particles require a smaller scale, such as was developed for measuring the wave length of light. Knowing that the micron is a thousandth part of a millimeter, and forgetting that its name and symbol were chosen to designate a millionth part of a meter, the symbol $\mu\mu$ was introduced to designate the thousandth part of a μ . The physicist, however, realizing the error in designating a thousandth part by μ , uses the symbol $m\mu$, the so-called *millimicron*, for the thousandth part of a millionth part of a meter, as m stands for a thousandth part. The physicist also uses the symbol $\mu\mu$ but correctly so, *viz.* to indicate the millionth part of a millionth part of a meter, the so-called *micromicron*.

It is impossible to grasp the true magnitude of such minute dimensions, but some idea of them can be gained if we approach them from things of appreciable size. The following table may help to do this:

1 meter (m.)	= 1,000 millimeters.....	Sound waves are 16 m. to 17 mm. in length
1 millimeter (mm.)	= 1,000 microns.....	Cells range from 0.15 mm. to 1μ
1 micron (μ)	= 1,000 millimicrons.....	Colloidal particles range between 0.1μ and $1 m\mu$
1 millimicron ($m\mu$)	= 1,000 micromicrons ($\mu\mu$)	Molecules range from $2.5 m\mu$ (protein) to $46 \mu\mu$ (water) and less

To this scale, each member of which is a thousand times the one below it, may be added the angstrom unit, A. U., used chiefly in indicating the wave length of light. It is $0.1 m\mu$ and therefore $100 \mu\mu$. The light waves of the visible spectrum are 7,500 A. U. long at the red end and 3,900 A. U. long at the violet end.

Particle size is of importance in the colloidal world in that it determines many essentially colloidal characters, such as surface and stability. To be truly colloidal, a dispersion must be permanent, yet permanency is relative. Gold particles which remain in suspension for five years are certainly colloidal, and particles that settle in five minutes are not. Obviously, no sharp line can be drawn between these limits. Clay particles that settle in five hours or five days are colloidal only in so far

as they, while in suspension, show colloidal properties. Atmospheric dust usually settles rather quickly, but some remains long suspended in the air. When the volcanic island Krakatoa blew up, the heavier pieces soon fell, but the finest of the dust particles traveled around the world. The particles of dispersed oil in the emulsions of our common experience are well above the lower limit of microscopic visibility, yet these emulsions are typically colloidal in that they show colloidal properties. So also is it with foams (*e.g.*, soapsuds and sea foam) the dispersed air bubbles of which are often far above ultramicroscopic (colloidal) dimensions, frequently even of macroscopic size, visible to the unaided eye. Can such systems, therefore, be colloidal? According to a definition based on size of particle, no, but emulsions and foams present the problems of colloidal chemistry. Consequently, a definition based on particle size, while useful, must eventually give way to one based on behavior, for of colloidal systems we ask not what they are but what they do. A substance which exhibits colloidal properties must be regarded as within the field of colloidal chemistry, even if certain other conditions, such as particle size, somewhat arbitrarily laid down, are not met.

Another problem dependent upon particle size is that of the scattering, or reflection, of light by colloidal particles. The lateral illumination of dust particles in a darkened room differs in one fundamental respect from truly colloidal examples of the Tyndall cone. In the latter case, the particles are not visible as such; they merely appear to be so, while the illuminated dust particles in the room are actually visible. This is true because the dust particles *reflect* the light, while the colloidal particles *scatter* it. In order for a particle to act as a mirror, it must be at least as broad as the wave it reflects; in other words, if a surface is to turn back a wave, it must be as large as the wave. The cliffs of Dover will turn back any wave, but a small stick placed at the ocean shore cannot reflect the waves. It breaks them up, or scatters them. This is what happens to light and sound waves when they strike a particle smaller than themselves. The scattering of light is a form of diffraction. Diffraction, or scattering, takes place whenever light impinges upon an edge or point. Diffraction gratings (parallel lines ruled on glass, as many as 1,000 to a centimeter)

cause a beam of light to be diffracted, or broken up, and multiplied many times over (Fig. 168). That colloidal particles do not give rise to reflected wave fronts but act as centers from which light disturbances spread out in all directions is easily proved by a comparison of dimensions. If a mirror must have a diameter at least equal to the size of the wave it is to reflect, then colloidal particles must have a diameter of about 0.5μ (5,000 A. U.) in order to reflect white light. The visible spectrum lies between 0.39 and 75μ (3,900 to 7,500 A. U.). The average wave length of white light is, therefore, about 0.5μ , which is five times the upper limit of size (0.1μ) of colloidal particles; consequently, the particles are too small to function as mirrors.

Now that we are concerned with colloidal particles, let us consider for a moment if the particle need always be an aggregate of molecules, that is to say, if a true particle need always be present. We shall later see that while one group of colloids, the suspensions (*e.g.*, colloidal gold), are characterized by having particles that are aggregates of molecules, the other group (*e.g.*, gelatin) need not *necessarily* be built up of aggregates; the molecules themselves, because they are very large, satisfy the conditions of particle size. Furthermore, in other systems, particles as such need not even be present for the system to be colloidal. Charcoal has colloidal properties, but neither it nor the substance that it has absorbed (with which its pores are filled) is dispersed, in the true sense, for both phases are continuous. We must, therefore, define colloids as systems in which one substance is distributed discontinuously or continuously in another continuous substance. The definition previously given is, however, the generally accepted one and characterizes nearly all colloidal systems. Furthermore, colloid chemistry has grown up on the basis of the particle concept, and, while there are exceptions, the particle is still the distinguishing feature in most cases. It is, however, important to remember that in colloidal systems, *exposed surface* is the significant thing, whether on a large molecule, a particle, or a continuous surface.

Color.—The Tyndall cone is often of a bluish tone. Colloidal smoke is blue. Light rain clouds are blue. Snow and ice have a distinct bluish tinge. A trace of soap (which is colloidally dispersed) gives a tint of blue to water. Blue eyes are due to a turbid medium; *i.e.*, the color is colloidal. This predominance

of blue color appears to be the result of selective scattering. When a beam of white light enters a colloidal dispersion, waves of all sizes impinge upon the particles, but they are not scattered in like proportion. If we return to our cliffs and stick at the seashore, it will be evident that the smallest waves will be scattered (broken up) best by the smaller obstacle. The same is true of light waves. The color of colloidal systems is a phenomenon involving a number of factors, and it is difficult to be certain which of these is primarily responsible in a specific case, but the rule that the smaller the particle the greater the scattering of short waves appears to be of general application. To this can be added the fact that the shorter the wave of light the greater the amount scattered. (Stated more specifically, the intensity of the scattered light is directly proportional to the square of the size of the particle and inversely proportional to the fourth power of the wave length.) In fine colloidal dispersions, blue, therefore, predominates, in both color and intensity.

There are a number of color phenomena in nature which may be wholly or partly due to colloidal properties, though other factors usually enter in. What these other factors are and to what extent they play a part in natural colors is not always known. The blue of the deep ocean and of the sky appears to be due primarily to factors other than colloidal ones. Deep water means clear water where only the finest particles are in suspension. If the color of the sea is in part colloidal, then blue would be the expected color. The color of the sky by day is ascribed to the scattering of light by molecular and not colloidal particles, while the color of the sky at twilight is very probably a colloidal phenomenon.

We view the setting sun along a path close to the surface of the earth where dust is thickest and particles largest. The rays scattered by these particles will be long ones; red and orange should, therefore, predominate, and blue be absent in sunsets. When the volcanic dust of the exploded island of Krakatoa reached the West Indies, halfway around the world, the whole sky there was an unusually brilliant red throughout the day, as in a sunset, owing to colloidal particles of relatively large size.

The interpretation of the color of metallic colloidal suspensions is difficult. As gold particles increase in size, the color of the

solution changes from yellow to orange, rose, red, violet, blue, purple, and black. The yellow color is not colloidal but ionic; it is the color of a true molecular solution; *i.e.*, it is not a structural color, as are purple and blue. The color of the Tyndall cone of gold dispersions changes from a faint, dull green to brilliant green, yellow, and orange. When made in the laboratory, colloidal gold may be red, purple, or blue; but why is not known. Small concentrations of electrolytes bring about different colors. Distilled water ordinarily gives blue colloidal gold when prepared by the Burton-Bredig electrical dispersion method. A weak solution of potassium bromide or iodide, for instance, will yield red colloidal gold.

An interesting fact in colloidal color but one difficult of interpretation is that nonmetallic, *i.e.*, electrically nonconducting, particles give different color effects from those given by metallic, conducting particles.

Occasionally, precipitates change color while forming; thus, when Fehling's solution is added as a test for sugar, a red precipitate is the expected result. Frequently, however, an orange, yellow, or yellowish-green precipitate is obtained. These color changes appear to be associated with differences in the size of the particle of the cuprous oxide formed.

Bancroft and his colleague Mason have made an extensive study of colloidal color, especially in birds and insects. They state that blue eyes owe their color to a turbid medium which is localized in the stroma. Increase in the size of the particles of the turbid layer accounts for lessened clearness of the blue with age. Pigmentation in the stroma may combine with the colloidal blue to give green, hazel, or brown eyes. The non-metallic blues in the feathers of birds such as the blue jay, the bluebird, the indigo bunting, and the kingfisher are structural (colloidal). No one has ever succeeded in extracting any blue coloring matter from any blue feathers. The structural blue is due to the scattering of light by minute air bubbles in the horny mass of the barb of the feather. Green feathers result when feathers which would be blue because of structure (pores) are overlaid by a transparent layer of yellow pigment (carotin). Metallic or iridescent colors, such as those of the peacock and the humming bird, present a more difficult problem; the question is still open as to whether they are structural or pigment colors.

Rayleigh believed the colors due to multiple thin films separated by air.

The blue color of the sky and the deep sea has been the subject of much discussion. Rayleigh suggested, and the theory is now generally accepted, that the blue of the sky is due to the scattering of the short blue rays of light by the gas molecules of the atmosphere. In other words, there is a *molecular* scattering of light comparable to that produced by colloidal particles. Raman discovered that molecular diffusion of light occurs also in water, and therefore the blue of the sea can be explained in this way. There is, however, evidence enough to support the contention that suspended, colloidal matter is largely responsible for the color of the sea, as it is for the blue of smoke, fog, snow, and many colloidal solutions.

Brownian Movement.—In the September, 1828, issue of the *Philosophical Magazine*, Robert Brown published the fact that he had observed in germinating pollen grains the trembling motion of particles measuring about 0.004 to 0.005 in. in size. He remarked that their motion resembled "in a remarkable degree the less rapid motion of some of the simplest animalcules of infusion." Thus did there arise in his mind the question whether or not this extraordinary activity could be a vital one. Since he had found it in living plants, he investigated twenty-year-old herbarium specimens, which could not possibly have any life in them, and found there the same motion of minute granules. He thought that these particles were possibly the "elementary molecules" of organic substances. But he disproved this speculation himself when he later found that "rocks of all ages including those in which organic remains had never been found yielded the molecules in abundance." We now know that what Brown saw was the active motion that bears his name, the *Brownian movement* of colloidal particles.

Molecules, because of their innate kinetic energy, are in an active state of movement. The movement is not a vibration but a hither-and-thither or zigzag one in which the molecules go from place to place within a relatively small region, though they may wander quite far, without ever returning to the original position. The motion is not to be confused with the diffusion of molecules in solution, though the one (Brownian movement)

is the cause of the other. The energy which keeps a particle larger than a molecule in motion is presumed to be the kinetic energy not of the particle itself but of the neighboring molecules of the surrounding liquid medium; these strike the larger particle on all sides and impart to it a movement similar to the kinetic motion of the molecules themselves. The movement of larger colloidal particles is obviously slower than that of smaller ones. The latter move so rapidly that it is impossible to follow them; their motion is a trembling one of small amplitude. The larger particles, which Brown saw, exhibit a dancing motion of greater amplitude. When a large number of colloidal particles is viewed with the ultramicroscope (or dark-field illumination), the picture resembles that of the Milky Way at night with the stars dancing and scintillating against a black background. Particles exhibit active Brownian movement only when below a definite maximum size and in a medium of sufficiently low viscosity. Particles larger than about 4μ show no motion in water. The maximum size of particles exhibiting Brownian movement in water could be taken as the upper limit of the colloidal state (thus including the majority of natural and commercial emulsions). The viscosity of the surrounding medium determines the amplitude of motion for a particle of given size. As glycerin is some eight hundred times as viscous as water, a particle suspended in it cannot move as freely as one suspended in water. In glycerin, one or two microns is the maximum size of the particle which can exhibit Brownian movement. The amplitude of the Brownian movement of included particles is thus an indication of the degree of viscosity of a liquid. (In addition to particle size and viscosity of the medium, the temperature of the latter and the density of the particles are factors.) Milk is a convenient substance in which to view Brownian movement. Nearly all degrees of amplitude are visible. Most of the emulsion particles of butterfat are in an active state of motion, but some are too large. Einstein has given a mathematical expression for the mean distance that a particle will move in a given time through a given (gaseous) medium (based on the gas constant, the temperature, the number of molecules in one gram molecule, and a factor depending upon the viscosity of the medium and the size of the particle). Langevin simplified the equation, and Perrin applied it experimentally.

In his book "Les Atomes," Perrin has given the path of colloidal particles in Brownian movement (Fig. 77).

This activity of colloidal particles is believed to be and indeed must be largely responsible for keeping them in suspension. It is, therefore, one of the factors upon which the stability of colloidal dispersions depends.

Practically all that has been said about Brownian movement so far is applicable to protoplasm, the particles of which are often in an active state of motion. The ultramicroscopic picture of

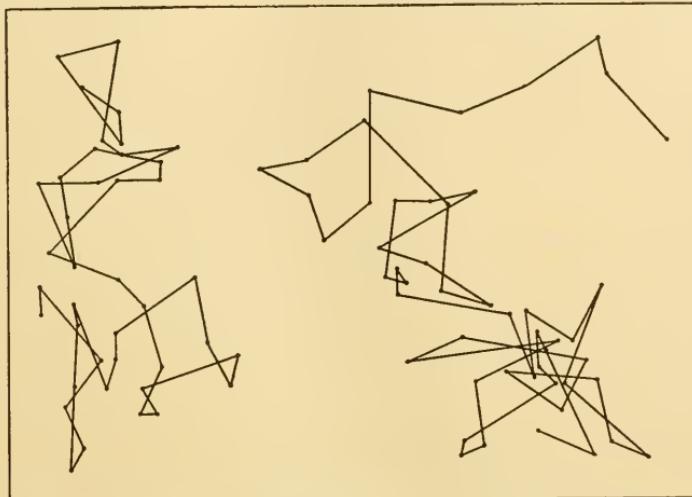


FIG. 77.—The path of colloidal particles in Brownian movement. (From Perrin.)

the plasmodium of a slime mold may also resemble the Milky Way with every star trembling. But it is not necessary to view protoplasm ultramicroscopically in order to observe the Brownian movement of its particles. In a slime mold or an amoeba, many particles may be in Brownian motion (apart from the movement due to the streaming of protoplasm). They may also at times be quiet, indicating a temporarily high consistency of the protoplasm.

Electrical Charge.—Colloidal particles possess an electrical charge similar to that of an ion; in fact, the particles may be regarded as colossal ions. Colloidal particles of platinum, gold, and silver are negative; lead, iron, and copper are positive. Among colloidal dyes, indigo and Prussian blue are negative; Magdala red and Bismarck brown are positive. Living "col-

loidal" particles such as bacteria, unicellular algae, mushroom spores, and blood corpuscles, and also products of living organisms such as fat droplets in milk, are all negatively charged.

We know that colloidal particles are electrically charged because they move in an electrical field toward one pole or the other, just as do ions. The nature of the electrical charge, of what it consists, and how it is held by the particle are difficult

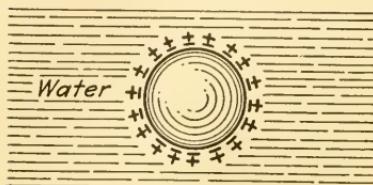


FIG. 78.—The Helmholtz electrical double layer on a colloidal particle.

problems. For the present, we shall limit ourselves to suspensions pure and simple, such as metal particles dispersed in water. The first constructive idea on the nature of the charge was advanced by Quincke and mathematically expressed by Helmholtz, who postulated the presence of two layers

of charges of opposite sign, adherent to the particle. These have come to be known as the *Helmholtz double layer* (Fig. 78). The charges could be adsorbed ions, possibly the positive hydrogen ions (H^+) and negative hydroxyl ions (OH^-) of water, but Helmholtz did not have ions in mind, as they were unknown at his time, nor does it now appear likely that H^+ and OH^- ions are responsible for the charge on metal particles.

Chiefly through the work of the Viennese medical colloid chemist Wolfgang Pauli, it seems probable that ions of salts of the metal in suspension formed at the time of (electrical) dispersion surround the particle.

A difficulty arose in regard to the Helmholtz double layer. If an equal number of charges of opposite sign surround a particle, they will leave the particle electrically neutral, but we know that colloidal particles are not neutral, for they wander in an electrical field. This difficulty was obviated by assuming that only the innermost layer clings to the particle and slips with the particle from under the outer layer, taking on other charges as the particle moves (Fig. 79).

Stability.—The stability of colloidal particles is in a great measure due to their electrical charge. As all the particles of a

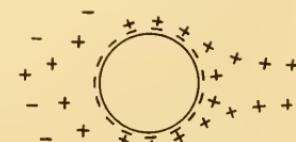


FIG. 79.—Manner of shedding the outer layer in the progress of a particle through its medium under the influence of a current.

dispersion are similarly charged, they will mutually repel each other and thus prevent settling. Should they be near collision, the layer of ions surrounding them serves as a protective coat and prevents coalescence. The presence of a protective layer, whether an electric covering of ions, a water mantle, or a membrane, is apparently necessary for the stability of colloidal particles. Experimental proof of it for colloidal metals is given by Pauli, whose work also indicates the chemical constitution of the ionic covering.

As it is necessary to observe absolute cleanliness in preparing colloidal solutions, because metallic suspensions are usually very sensitive to electrolytes, workers were led to believe that a gold suspension can exist only when pure gold is dispersed in pure water; from this opinion, others dissented. Duclaux suggested that metallic colloidal particles are surrounded by a field of ions from salts of the metal. Pauli explained and interpreted this idea by work which proved that pure gold dispersed in pure water does not remain in suspension. When the conductivity of the water is below $3 \text{ by } 10^{-6}$ (reciprocal ohms), and when an absolutely clean gold-plated vessel and pure gold electrodes are used, no good dispersion results; but if a trace of salt or acid is added to the water before the metal is dispersed, the suspension stays up. It is possible to make metallic dispersions without the addition of a trace of electrolyte only because the average laboratory-distilled water and the glass vessels used supply impurities enough to give an environment of adsorbed ions to the colloidal particles. The manner in which the electrolyte functions is as follows: When gold is electrically dispersed in a very weak solution of hydrochloric acid, the acid and gold combine at the arc to form gold chloride ions and hydrogen ions. The former adhere closely to (are adsorbed by) the gold particles and give to them their characteristic negative charge. The hydrogen ions are free in the water (Fig. 160). Whether the adhering ions are AuCl_4^- or AuCl_2^- was not readily determined, but Pauli now believes them to be AuCl_2^- , enveloped in a cloud of free H^+ . In an alkaline medium, the adhering ions are Au(OH)_2^- , and the free ions K^+ . In the case of an iron hydroxide dispersion, the situation is similar (Fig. 80) but more complex, for it is not certain whether the core of the particle is Fe(OH)_3 or, more likely, $(\text{FeOCl})_x$ (see pages 364, 367).

Numerous factors influence the stability of colloidal particles to a greater or lesser degree. Charge is the primary one. In the case of most solid (metal) suspensions, it predominates, but in the case of organic and certain inorganic suspensions, hydration is also an important factor in stability, though charge is effective here too. The maximum charge necessary is variable. Northrop and de Kruif found 15 mv. to be the critical potential (see page 376) of certain bacteria; but under other conditions (*e.g.*, when Na_2HPO_4 is present), the potential is higher. Other bacteria may remain in suspension

when their potential is reduced to zero. Here, hydration is probably responsible, as in the case of proteins. Still other strains of bacteria, studied by S. Mudd, also show zero potential, though stable over a wide range in acidity; this can mean only an unusual type of surface, possibly a carbohydrate. Stability would here be due to hydration.

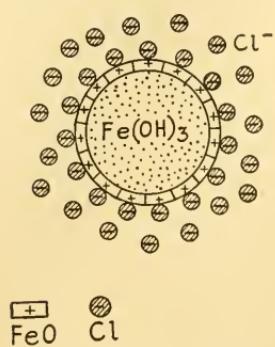


FIG. 80.—The ionic environment of a colloidal particle. (After Pauli.)

movement, absence of cohesion, convection, and size; of minor significance are shape, and temperature.

Precipitation.—The formation of deltas at the mouths of rivers is an example of the precipitation of colloidal matter. When the muddy waters of the river reach the sea, the fine clay particles in suspension are precipitated by the salts of the sea, and deltas result. If salt or other electrolytes are added to a colloidal solution in a test tube, precipitation ordinarily takes place. If a colloidal suspension of negative charge, such as silver, is added to one of positive charge, such as iron hydroxide, both are precipitated. The opposite charges have neutralized each other. Two positive or two negative colloids do not (usually) precipitate one another. Precipitation by electrolytes is due to neutralization of the charge on negative colloidal particles by positive cations (Na^+ , Ca^{++} , Al^{+++}), and of the charge on positive colloidal particles by negative anions (Cl^- , SO_4^{--} , PO_4^{---}). Precipitation and the closely related processes of coagulation,

agglutination, etc., are of such biological importance that they will be considered in more detail later (page 479). We may, however, here ask an apparently simple question; if one ion of the precipitating salt is carried down with the precipitate, what happens to the other? It can hardly be left in the solution uncompensated for. Either it must also be carried down (in a purely passive way with the coagulum), or it is balanced by an equivalent amount of oppositely charged ions possibly set free from the surface of the particle.

The theory of colloidal precipitation is based on the electrical neutralization of charges. But if a particle can be decharged in some other way, the result will be the same. Two positive or two negative colloids may precipitate each other. They probably do so through chemical interaction of their stabilizing ions. The ionic environment of one kind of particle (negative colloidal sulphur) may react with that of another colloid (As_2S_3) of the same sign, so as to eliminate the ionic environment; thus is the particle decharged, not physically (electrically) but by a chemical reaction.

Some interesting attempts have been made to apply colloidal theories of precipitation to the elimination of fog, smoke, and obnoxious fumes. English and French scientists considered the possibility of dispelling fogs by sending powerful hertzian waves (magnetic oscillations resembling light waves but much longer) out into the air. Another application of this method is found in a process now in use in the California oil fields for separating emulsified (colloidal) water from crude oil. Efforts have been made to handle the smoke nuisance of large cities in this way; but the most successful application of the method is to gases in smelter flues. The method is based on the fact that if a needle point and a flat plate are connected to a high-potential current, the air between them becomes highly charged with electricity of the same sign as the needle point. Any body brought into this space instantly receives a charge of the same sign. If this body is free to move, as in the case of a floating particle, it will be attracted to the plate of opposite charge. Plates attached to the sides of smelter flues serve as the plate electrode. The point electrodes are the fine tips of asbestos fibers; these are more effective than wire and proved to be the key to the first commercially successful installations. Another application of precipi-

tation by electrical discharging, but one which never got beyond the speculative stage, is that suggested by Bancroft, who had the idea that fog over airplane landing fields could be dispersed by the scattering of charged sand from the airplane. The plane would carry sand which could be electrically charged and spread over the fog-covered field just before landing. The suggestion is a fertile though impractical one, but it may lead to a similar, yet commercially feasible, solution of the problem.

The Suspensions.—If the conditions of colloidality are present when one kind of matter (gas, liquid, or solid) is finely divided and permanently suspended in another kind, then there should be nine colloidal types. All but one of these exist, the exception being gas dispersed in gas, which is always a molecular and never a colloidal dispersion. The eight types of colloidal suspensions are:

Gas in liquid	Liquid in solid
Gas in solid	Solid in gas
Liquid in gas	Solid in liquid
Liquid in liquid	Solid in solid

Gas-in-liquid colloidal systems are foams. They are of frequent occurrence in nature and present some important commercial problems, such as the lather-forming qualities of soaps. It should be noted that while foams of gas dispersed in a *pure* liquid exist, they are rare and unstable. All naturally formed *gas-in-liquid* systems, and most artificially made ones, contain a third phase which surrounds and stabilizes the dispersed gas bubbles. (We shall later see how this third phase functions.)

Gas-in-solid systems belong chiefly to that class of colloids in which both phases are continuous. These systems are more typical of the second group of gel-forming colloids than of the suspensions where we now put them. Pumice stone, charcoal, and air-filled silica gel, the latter two of commercial value as adsorbents, are examples.

Liquid-in-gas systems, in the form of fogs and clouds, give the meteorologist colloidal problems to think about. When air rises to higher altitudes and lower temperatures, the invisible molecules of water vapor which it contains aggregate to form colloidal particles of water, and a cloud results. The cooling of the air at night has the same effect and forms early-morning mist.

As the progress of aggregation goes on, the colloidal droplets coalesce to form larger drops of rain or dew. (Coalescence follows condensation which takes place on dust or other particles in the air. Both condensation on particles and subsequent coalescence are colloidal phenomena.) A miniature cloud is formed when steam is set free in a cool room.

Liquid-in-liquid systems are emulsions; they hold a prominent place in our everyday life. The most familiar example is milk, an emulsion of butterfat dispersed in water (with salt, sugars, casein, etc.). Medicinal emulsions of vegetable and mineral oils have now become common. Emulsions may be of oil dispersed in water or water dispersed in oil. The former are usually of relatively low consistency, as is milk, while the latter are usually firm enough to hold their shape unsupported. Cold cream and mayonnaise are water-in-oil emulsions.

Liquid-in-solid systems arise when the pores of gas-in-solid systems (dry clay, charcoal, silica gel) become filled with liquid. Wet clay is generally regarded as a liquid-in-solid colloid, but Freundlich calls attention to the fact that in clay, the water may be the continuous phase and the clay discontinuous, which is the condition characteristic of solid-in-liquid systems. This is a likely point of view, as clay particles are separated from each other by thin films of water which surround them. Knowledge of the colloidal behavior of clays is the means toward a solution of the problems of soil moisture. Possibly more truly of the liquid-in-solid type are the pearl and opal. The opal is chiefly silica (sand) and water. Pearls are mostly calcium carbonate (marble) and water. If pearls are kept in a very dry place, as in a safe deposit box, for a long time, they lose their "life," or luster, owing to the loss of their colloidal water. They retain their beauty best when worn frequently next to the skin, because they are then in a moist atmosphere.

Solid-in-gas systems are of interest because of atmospheric problems. The precipitation of smoke and other air impurities, such as fumes in cities, is a colloidal problem involving solid-in-gas systems. Smoke that quickly settles is a coarse dispersion of carbon in air; the "blue haze" of forest fires and of tobacco smoke is more truly colloidal, as it remains long in suspension. Atmospheric dust, miles above the earth, is a solid-in-gas colloidal system. To it has been ascribed the blue color of the sky, but,

as we have seen, this may be due to gaseous molecules rather than to colloidal particles.

Solid-in-liquid systems are of great variety and importance. Our first example of the colloidal state was a solid-in-liquid system, *viz.*, the muddy waters of the Mississippi. Solid-in-liquid suspensions are met with in the laboratory in the form of dispersions of metals, such as gold, silver, copper, etc., in water. It was a dispersion of gold in water which Faraday, in 1886, showed to the Royal Society. The solid-in-liquid systems are the chief representatives of the suspension colloids which in many respects differ so prominently from the gluelike (jelly-forming) colloids.

Solid-in-solid systems are met with in nature as blue rock salt (sodium in sodium chloride), black diamond (minute diamond crystals separated by amorphous carbon or graphite), and precious stones. The last owe their color, in part, to impurities colloidally dispersed in them, *e.g.*, the emerald to chromium and the topaz to iron. Such systems are produced commercially as colored glass; ruby glass is metallic gold dispersed in glass. It is not a difficult experiment to make solid-in-solid colloidal systems and imitate the precious and semiprecious stones in nature. If a small amount of a dilute solution of gold chloride is added to crystals of any convenient salt, such as sodium chloride or potassium bromide, and the mixture brought into a state of flux by heating in a porcelain crucible and allowed to cool slowly, a colloidal dispersion of gold in the crystalline salt results.

The tremendous importance of colloidal chemistry can be realized from the fact that not only natural processes such as weather conditions, muddy river water, the formation of deltas, and soil problems in agriculture but also commercial processes such as the manufacture of artificial silk, photographic negatives, paint, gelatin, cheese, medicinal and other emulsions, dyes, paper, and ceramics, whether involving the casting of a brick from cheap clay or the molding of the finest porcelain from kaolin, are all problems in colloidal chemistry. But such substances are not our chief concern here, and therefore it is not in their colloidal behavior that we are primarily interested. A basic knowledge of colloid chemistry is necessary in order to interpret the properties of protoplasm. Superficially, protoplasm is a fine emulsion, a liquid-in-liquid system. In its finer

ultramicroscopic structure, protoplasm is in part a system of the second class of colloids, the gluelike ones and in part a true solution of salts, sugars, etc.

Classification.—We have devoted our attention in the present chapter primarily to colloidal suspensions of solid matter, with only a brief reference to liquid suspensions (emulsions) and glue-like colloids. In order satisfactorily to classify, we must have an intimate knowledge of all types of colloidal systems. Still, a premature attempt at classification here will help toward a better understanding of what is to follow.

Those colloids that resemble glue have several properties which sharply distinguish them from suspensions of solid and liquid matter. One of these is the capacity to form *gels*, or *jellies*. Gelatin is the classical example among the gel-forming colloids, but there are many others of great variety. They include albumin, hemoglobin, casein, rubber, vegetable gums, agar, soap, glue, cellulose, silica gel, and such extreme types as glass and mineral gels. The distinguishing feature of nearly all of these is their ability to take up water. In doing so, most of them *swell* and are therefore said to *imbibe* water. Graham called these systems *gels* (the first syllable of "gelatin") when they are in the firm condition, and *sols* (the first letters of "solution") when they are in the liquid condition. The latter term also applies to suspensions of solids, *e.g.*, a gold sol. Gels are usually glutinous (sticky) and elastic, though there are some nonglutinous and nonelastic ones. They coagulate readily, as do blood, casein (in milk), and albumin (in egg). They are not very sensitive to electrolytes and are therefore precipitated much less readily by salts than are the suspensions. Among the very few properties that they have in common with the suspension colloids are their failure to dialyze, and the exhibition of the Tyndall cone. It is quite evident, therefore, that there are two major groups of colloids which differ greatly—the suspensions and the gel-forming ones. On the basis of their differences, they can be named. It is typical of gels to take up water. The suspension colloids lack this ability and have, therefore, been called *hydrophobic* ("water hating"), while the gel-forming colloids are *hydrophilic* ("water loving").

The German colloid chemist Herbert Freundlich, perhaps the best known of all workers in the colloidal field, thought that since

the liquid imbibed need not always be water, the term should be more general. He therefore proposed *lyophobic* and *lyophilic* (Gr. *lyos*, solution). These names are now the most generally used. Numerous other names have been suggested. They indicate the many ways in which the two groups can be distinguished.

Lyophobic colloids	Lyophilic colloids
Hydrophobic colloids	Hydrophilic colloids
Anhydrophilous colloids	Hydrophilous colloids
Irreversible colloids	Reversible colloids
Typical or genuine colloids	Baser colloids
Unstable colloids	Stable colloids
Electrosensitive (electrocratic) colloids	Electroresistant colloids
Suspension colloids	Emulsion colloids
Suspensoids	Emulroids
Nongelatinizing type	Gelatinizing type
Colloidal suspensions	Colloidal solutions

The colloidal systems (lyophobes) referred to in the foregoing left-hand column are of three major kinds—solid, liquid and gaseous suspensions (metal sols, emulsions, and foams). Subgroups under the lyophilic systems include the *turgescent* and the *nonturgescent* gels, that is to say, gels which swell and those which do not. Gelatin is a turgescent or swelling gel, while silica gel is nonturgescent. The former is elastic, while the latter is (relatively) inelastic. They differ also in that the nonturgescent type becomes porous on drying, while the turgescent type does not. In view of the fact that the swelling or turgescent gels (gelatin) are elastic and nonporous, while the nonswelling or nonturgescent ones (silica) are nonelastic and porous, it does not matter which property we use upon which to classify them, for it is the same in the end, and our choice really rests on that property in which we are primarily interested.

Any classification of colloids built on physical properties is, in the present state of our knowledge, certain to be an artificial one. The lyophobes (suspensions of metals or oils) present, in the main, a natural group. The lyophiles, on the other hand, are an unnatural and heterogeneous collection, natural subgroups among them being the soaps, the carbohydrates (agar, dextrin, cellulose), and the proteins. Zsigmondy evolved a classification based on chemical rather than physical properties. While

helpful, it is not colloidal, for colloid chemistry has grown up on a physical and not on a chemical basis.

The following is Zsigmondy's classification:

I. Inorganic		II. Organic	
A. Metals		A. Organic salts	
1. Pure		1. Soaps	
2. With protective colloids		2. Dyes	
B. Nonmetals (colloidal sulphur)		B. Proteins	
C. Oxides (iron oxide, silica gel)			
D. Sulphides (arsenic trisulphide)			
E. Salts (silver salts of photography)			

Zsigmondy's groups of colloidal systems lack the emulsions and such organic gel-forming colloids as agar, starch, and cellulose. These latter might be included under "carbohydrates," between the organic salts and proteins.

Victor Cofman has devised a classification in which both physical and chemical properties are considered. The plan has the good feature of indicating the overlapping of types, by taking into consideration the imperceptible graduations which occur between one kind of system and another. Cofman's classification is the following:

Heterogeneous systems				Homogeneous systems			
Physical mixtures		Physical compounds			Chemical compounds		
Coarse dispersions	Fine (colloidal) dispersions	Solutions	Colloidal compounds (elastic gels)	Hydrous oxides; oxy-salts	Hydrates and alloys	Dissociable compounds	Nondissociable compounds

One might well ask, is there, among all these diverse types of systems, one property which definitely stamps the colloidal state? The answer is, yes, the proportion of surface to volume. Colloidal systems owe their distinctly colloidal properties to the extraordinary amount of surface exposed. When the exposed surface of a dispersed substance is very great in proportion to the total amount of dispersed substance present, *i.e.*, when a substance is in the colloidal state, then surface phenomena determine the behavior of the system. Spongy (colloidal) platinum is very

active. Metallic (solid) platinum is relatively inert. The physics and chemistry of colloids are the physics and chemistry of surfaces. Some conception of the extent of this surface can be gained by considering the increase in surface which takes place when a cube 1 cm. on an edge is subdivided.

Size of cube, edge in centimeters	Number of cubes	Total surface, square centimeters
1.	1	6
0.1	1,000	60
0.01	1,000,000 (10^6)	600
0.001	10^9	6,000
0.0001	10^{12}	60,000
0.00001	10^{15}	600,000

The same fact can be expressed by stating that if 1 cc. of water is sprayed into droplets 0.01 μ (0.00001 mm.) in diameter, the total area will be 6,000,000 sq. cm.

While matter in the colloidal state owes its unique properties to the tremendous surface exposed, there are other properties which rather definitely characterize colloidal systems. The most general of these is turbidity, as shown by the Tyndall cone. The colloidal particle has also served as a basis of definition. The word "particle" suffices for suspension (lyophobic) colloids, but in gluelike (gel-forming lyophilic) colloids, the structural unit is assumed to be of a special nature and has been given the name *micella* or *micelle*. This term, with the theory of structure underlying it, was the fundamental contribution of the German botanist Karl von Nägeli. Nägeli believed that jellies are built up of discrete units larger than the molecule but too small to be seen by direct microscopic observation, in other words, of colloidal particles somewhat similar to those which characterize solid suspensions. He believed the micelle of gels to be a minute crystal. Present-day chemists, being of a similar opinion, have called it a *crystallite*. The term micelle is generally reserved for the structural units of gels and not usually applied to the colloidal particles of suspension colloids. The micelle is characteristic of colloidal systems, but it may not be a necessary distinguishing feature. Small size of the structural unit when compared to visible particles and large size in comparison to the average

molecule are the bases of the unique behavior of colloidal systems. The micelle satisfies these qualifications, for it is an aggregate of molecules and below microscopic visibility. When a molecule (such as those of proteins) is large enough to present an appreciable surface, then it will qualify colloidally as well as does a micelle.

Biological Applications.—Protoplasm, in its coarser microscopic structure, is a suspension of minute particles; it is, therefore, a suspension (lyophobic) colloid. The matrix or dispersion medium (continuous phase) of this suspension contains gel-forming substances (albumin, etc.) which impart to protoplasm the properties of gels or jellies. These substances and their properties are believed to be more fundamental in life than the superficial and microscopically visible colloidal suspensions; however, both play their part, both are necessary to the vital activities of protoplasm.

The colloidal state of protoplasm will be considered again and again in this book. We may here add one application of the suspension colloids in medicine. This is in the field of antiseptics. Heavy metals, such as copper and mercury, are highly toxic. Colloidal preparations of metals, particularly silver, dispersed in water or oil or prepared in dry form to be later dispersed by the user, have come into extensive use as antiseptics. Good results are claimed for colloidal silver in the treatment of throat, nose, and sinus inflammation and in bladder troubles (*cystitis*), but the curative powers may in many cases be purely palliative. Assuming that the beneficial results are due to the germicidal action of the solution, upon what do these peculiar properties rest? The high toxicity of heavy metals suggests that the metal itself is the agent. Freundlich and Sollner found that colloidal silver is taken out of suspension and deposited within algae. They believe this to be the explanation of the oligodynamic effect which Nägeli obtained for copper (page 428). Precipitation of the bacteria by neutralization of their electric charge is not a satisfactory interpretation of the toxic effect of colloidal metals, as both silver and bacteria are negative in sign. While it is possible that the metallic solution may owe its germicidal qualities to the toxic effect of salts of the metal, it is also possible that instead of the metal being deposited within the cells, the bacteria are coated, literally plated, with a layer of

the metal. It is said that only metals which do not unite with oxygen directly, and are therefore stable in the presence of tissues, are germicidal; silver, gold, and platinum would, therefore, qualify, and copper not, though copper is highly toxic and was the first oligodynamic metal. Colloidal gold has been introduced as a germicide for use in tuberculosis.

Conclusion.—The colloidal world is unique. It has, in the main, its own laws. Classical laws formulated for gases, liquids, and solids may or may not apply to colloidal systems, and when they do apply, they usually do so only in a modified form. That property primarily responsible for colloidal behavior is surface. We have seen how great the increase in surface may be when solid matter is finely dispersed. With this tremendous increase in surface, the system takes on new and unique properties; thus, capillarity and Brownian movement belong to the colloidal world alone. If the dispersion is carried too far—to that of molecules—the system ceases to be colloidal, and new properties again characterize it, *viz.*, those of solutions. We must, therefore, be cautious in applying the known laws of one kind of system to another distinctive system. For example, colloidal particles and the bodies of the solar system have much in common; both are freely suspended and both possess an electric charge on their surface; yet how different are the laws that govern them. In the former case, gravity predominates, while electric forces, important to us who live upon the earth (*e.g.*, as lightning), are to all other (celestial) purposes nonexistent, and the astronomer pays no attention to them. In the colloidal world, the situation is reversed; surface charge is the significant factor, and gravity is a negligible quantity. The two systems are superficially alike—they look alike when one is seen with the naked eye and the other with a microscope—and they have certain properties in common, yet they are two distinct worlds; because of this, they acquire new properties and are governed by different laws.

CHAPTER VII

EMULSIONS

Viewed through the microscope, protoplasm presents the picture of an emulsion—a colloidal dispersion of fats and like substances in an aqueous medium. Whether or not we regard this visible structure of protoplasm as fundamental, it nevertheless plays a significant part in the life of the cell so that a knowledge of it, and therefore of emulsions in general, becomes most important for an understanding of protoplasmic behavior.

An *emulsion* is a system in which one liquid is finely and permanently dispersed in another with which it is not miscible. Very dilute emulsions of oil and water alone are model suspension (lyophobic) colloids; that is to say, they have the properties typical of solid (metallic) colloidal suspensions. Concentrated emulsions, on the other hand, have certain (electrical) properties in common with colloidal suspensions but also certain (viscous) properties in common with the gluelike or lyophilic colloids. For reasons that will become obvious later on, the several lyophobic characters of emulsions are more significant than is the one lyophilic property; consequently, the emulsions are classed as lyophobes.

Emulsions, as ordinarily met with commercially and in nature, are of oil and water. The oil may be dispersed in water, or the water dispersed in the oil. Milk is an oil-in-water emulsion, and cold cream a water-in-oil one.

Pure emulsions of oil and water alone can be made only if one of the phases (ingredients) is less than 1 part in 100 parts of the other. Such emulsions are truly colloidal, in that the dispersed particles are of ultramicroscopic size. The common emulsions are more concentrated, having nearly equal proportions of oil and water, and the dispersed particles are often relatively large. All such emulsions must be *stabilized* by a third substance—the *stabilizer*, or *emulsifier*—which is usually a lyophilic colloid, such as gelatin or soap. This third phase plays its role as stabilizer by forming a membrane around the dispersed globules. When

milk is churned, the protective membrane is broken, and the fat globules coalesce, forming butter. Among the numerous constituents of milk, which include water, butterfat, salts, sugars, albumin (lactalbumin), casein (caseinogen), and phosphatides (lecithin), any one of the last three could function as the stabilizer. Lectalbumin or caseinogen has usually been regarded as the emulsifying agent in milk. The fact that the fat globules in milk cannot be easily stained with Sudan III, which is an oil-soluble dye, suggests that the membrane is protein, but it is believed by some that the membrane material in milk is chiefly phosphatide, a nitrogenous fat, rather than a protein. Perhaps the stabilizer is a complex of fat and protein; caseinogen is itself a phosphoprotein.

Newer technique involving cataphoresis (page 361) gives an ingenious method for ascertaining, with a fair degree of certainty, the nature of the covering on colloidal particles. Because of the charge which they possess, colloidal particles travel in an electrical field. They reverse their charge, or, rather, they are at zero charge, at the so-called "isoelectric point," which is often expressed in terms of the acidity (pH) of the solution. This point of zero charge is very specific. A substance can be characterized by its isoelectric point or point of no migration in an electrical field. If the isoelectric point of milk globules is at a pH, or acidity, value which is specific for protein and not near the isoelectric point of phosphatides or other fatlike substances, then it is likely that the coating on the fat globules is of protein. The pH value at which the suspended particles in milk show no movement in an electrical field is 4.6, which is the isoelectric point of casein. On the basis of this evidence, it would seem that the coating on fat globules in milk, *i.e.*, the stabilizer, is protein rather than some other substance.

The stabilization membrane quite completely isolates the dispersed phase of an emulsion from the dispersion medium. It should, therefore, be possible to emulsify two miscible substances, such as an oil in an oil, if the stabilizer could be got around the dispersed oil before it is put into the dispersion medium. This has actually been accomplished with casein for the membrane.

The distinguishing properties of colloidal systems reside primarily in the interface. Nowhere is this more true than in

emulsions. The type, permanency, consistency, and reversibility of emulsions are determined by the interface—the stabilization membrane.

It is quite an easy task to make an emulsion. If the oil and emulsifier are suitable, mere shaking in a flask is sufficient. Emulsions so made usually separate, or "break," in a short time. More thorough churning is necessary to make a good stable emulsion. This is accomplished either by systematic shaking or preferably, by *homogenizing* in a *homogenizer* or *colloid mill*. Commercial emulsions are made with the aid of a colloid mill which forces and scatters the oil through a fine nozzle under high pressure. So-called "artificial milk" can be made in the laboratory by grinding gum arabic, distilled water, and butterfat (melted butter) in a mortar until the oil is well emulsified; the mixture is then diluted, and synthetic milk is the result. Milk and cream are produced by emulsifying butterfat in a solution of skimmed-milk powder.

Emulsions are of two types—oil in water (*a*, Fig. 81) and water in oil (*b*, Fig. 81). It is not always possible to say which type will result when oil and water are shaken together with an emulsifier; thus, one petroleum oil—kerosene—forms an oil-in-water emulsion with casein as the stabilizer, while another petroleum oil—Nujol—forms a water-in-oil emulsion with the same stabilizer. The reason is unknown.

There are a number of ways of ascertaining the type of an emulsion; for example, the water-in-oil ones are usually much thicker than the reverse type, as cold cream and milk illustrate. The most reliable way to distinguish the two types is to examine them under the microscope; such observations are aided by staining one of the phases before making the emulsion (the oil with Sudan III). The color so produced serves in another way to distinguish the two types; if the red-stained oil is the outer, continuous phase (the dispersion medium), the emulsion as a whole appears red; if the red oil is the dispersed, or discontinuous, phase, the emulsion is pink (Fig. 82). Another method is to observe the walls of the glass container after shaking the emulsion; if the glass surface is left fairly free from oil, then water is the outer, continuous phase; but if the walls of the container are oily, then the oil is the continuous phase. A novel method is to pass an electric current through the emulsion; if 10 to 100

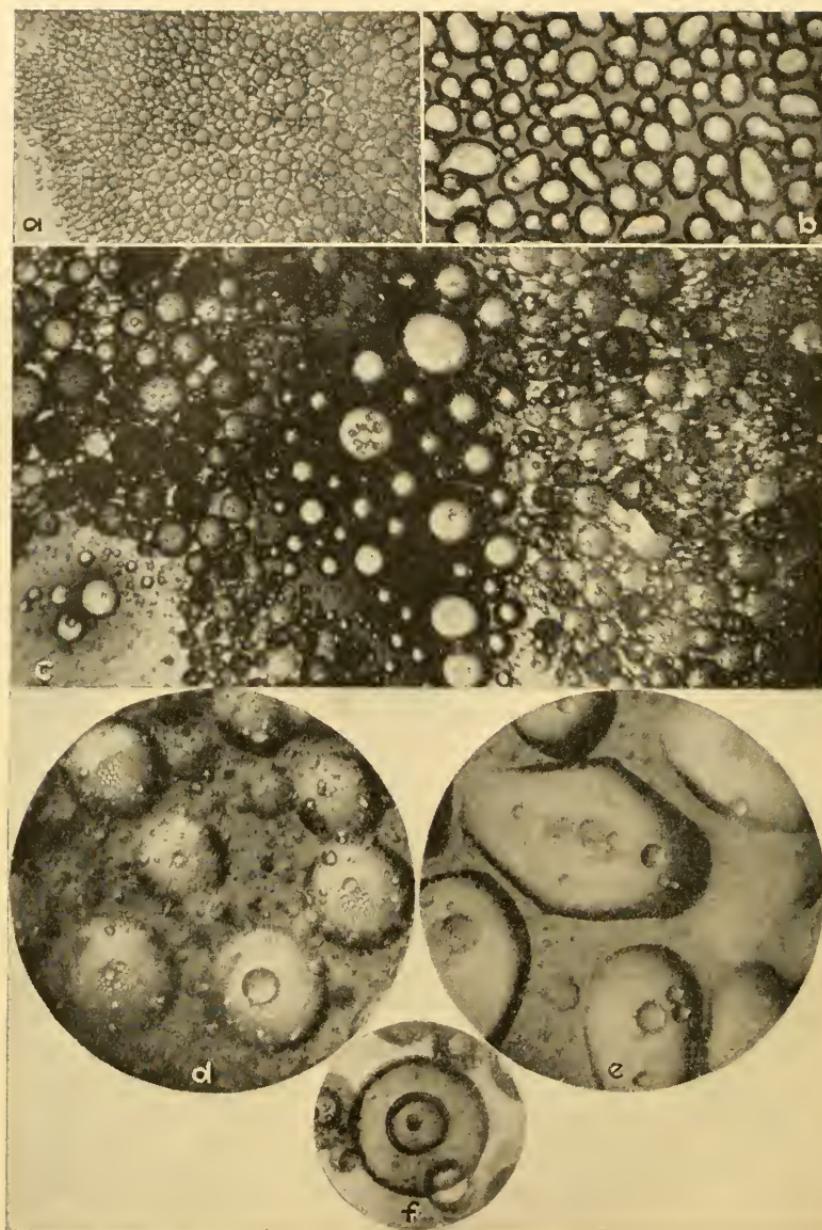


FIG. 81.—(a) An oil-in-water emulsion; (b) a water-in-oil emulsion; (c) both types in one sample, with one water globule (center) containing an oil-in-water emulsion; (d) a coarse water-in-oil emulsion, with the large water globules containing small amounts of oil-in-water emulsions preparatory to going over into the latter type; (e) a very coarse water-in-oil emulsion just before breaking or reverting to the opposite type; (f) five emulsions in one (consisting of one globule each), starting with the smallest oil drop in the center, the emulsions are OW, WO, OW, WO, OW.

milliamperes go through at a voltage of 110, the emulsion is an oil-in-water one, because the water, with its electrolytes, is the continuous phase and acts as a fair conductor; if no current passes, then oil, a noneconductor of electricity, is the continuous phase, and the emulsion is a water-in-oil one.

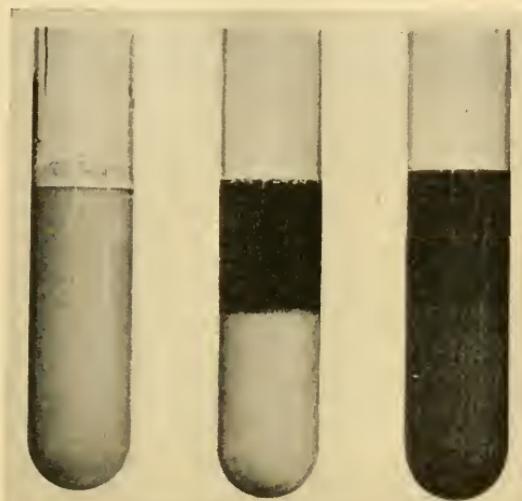


FIG. 82.—An oil-in-water (left), a "broken" (center), and a water-in-oil (right) emulsion in bulk; the oil phase is stained red, therefore the left sample is pink, the center one, red above and white below, and the right one red.

Phase Reversal.—Most emulsions can be reversed from one type to the other—from oil in water to water in oil, and vice versa. The addition of a suitable electrolyte (salt, acid, or base) will usually do it. If the emulsion is one of olive oil in water, stabilized by the soap sodium oleate, it can be reversed by adding a little calcium chloride. The water-in-oil system thus formed can now be thrown back to the original oil-in-water type by adding sodium hydroxide. Under favorable conditions, reversals may be repeated eight or ten times. While some emulsions can be frequently reversed, others will not budge. The above olive-oil emulsion reverses readily if soap or casein is the emulsifier but refuses to change type if albumin or saponin is used. Why, it is at present impossible to say. It is possible that presumably irreversible emulsions could be made to change type if the right electrolyte or other agent were found. As the membrane is the seat of the mechanism of reversal, it is also possible that the substance of which it is formed, *i.e.*, the emulsifier, is pre-

cipitated or coagulated at the oil-water interface and thus forms a film which is not readily, if at all, influenced by electrolytes.

The oil phase plays a part in determining the kind, stability, and reversibility of an emulsion only in so far as it helps determine the type of stabilizing membrane. Kerosene and Nujol oil give opposite types of emulsions with the same stabilizer.

The role of the oil in determining the type of an emulsion is evident in the interesting behavior of hydrocarbon oils of different densities. If such oils are emulsified in water, with casein as the stabilizing agent, the light oils (hexane and gasoline) all form stable oil-in-water emulsions. Slightly heavier oils form emulsions of the same type, but they are unstable. The medium (light lubricating) oils either do not emulsify at all or form mixtures of both types; they lie in the zone of reversal. Slightly heavier oils form emulsions of the reverse type—water in oil—but they are unstable. The heaviest (heavy lubricating) oils form stable water-in-oil emulsions. This information is tabulated in the following table:

Name of oil	Specific gravity	Type of emulsion	Texture	Stability
Isohexane.....	0.664	O W	Fine	Stable
Isooctane.....	0.726	O W	Fine	Stable
Kerosene.....	0.820	O W	Medium	Moderately stable
Distillate.....	0.828	O W	Coarse	Unstable
Mineral seal.....	0.849	Separates immediately
Distillate.....	0.857	W O	Medium	Moderately stable
Paraffin oil.....	0.884	W O	Fine	Stable
Cylinder oil.....	0.918	W O	Fine	Stable

It has not been possible to give an explanation of this behavior of hydrocarbon emulsions on the basis of existing theories of emulsification.

The process of changing over, when observed under the microscope, reveals some of the details of the mechanism of phase reversal. At the onset of the collapse of an oil-in-water emulsion, the dispersed oil globules become irregular in shape and grow in size by the coalescence of several smaller globules (Fig. 83). The now larger oil globules become attenuated (pear-shaped) owing to reduced surface tension at the one, pointed end (*e*,

Fig. 81). Finally, they open up at the end of low surface tension, pouring out their contents, which form the dispersion medium of the new water-in-oil emulsion. In the meantime, the water, the former continuous phase, has been breaking up into droplets during the process of shaking. This change has been diagrammatically represented by Clowes (Fig. 83).

Compound Emulsions.—In the process of reversal with electrolytes, there is seldom a clean separation of oil and water at the reversal point; that is to say, one type is always being formed before the other type is fully done away with; consequently, instead of the oil and the water being separated into two layers at the reversal point, both types of emulsions usually exist side

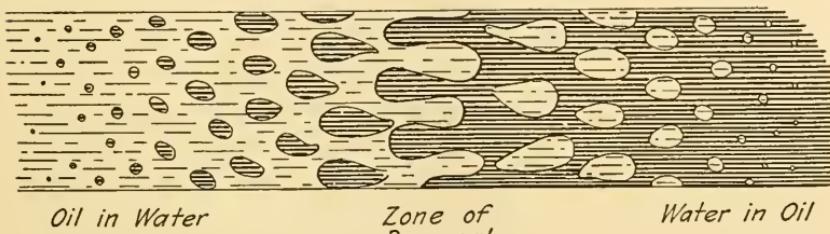


FIG. 83.—Diagrammatic representation of phase reversal in an emulsion. (*Modified from Clowes.*) At the extreme left is a fine oil-in-water emulsion; the globules become larger, then pear-shaped (left center), finally collapsing (center) and emptying their contents (zone of reversal); the same takes place in the water-in-oil emulsion (right) when reversing. The several stages given here diagrammatically are fairly well illustrated in Fig. 81 where *b* is a fine water-in-oil, *d* a coarse water-in-oil, and *e* the large pear-shaped water globules about to open up to form the continuous phase of an oil-in-water emulsion.

by side and one within the other (*c, d*, Fig. 81). Many interesting things are to be seen in emulsions at or near the reversal point. An oil globule, which is part of an oil-in-water emulsion, may itself be a water-in-oil emulsion. This situation may reach the extreme case of five emulsions, one within the other, like a chest of Chinese boxes (*f*, Fig. 81).

Theories of Emulsification.—Two hypotheses have been advanced in an attempt to explain the behavior of emulsions—the solubility, or surface-tension, hypothesis of the American colloid chemist Wilder D. Bancroft, and the oriented molecular-wedge hypothesis independently developed by the California chemist J. H. Hildebrand and the Chicagoan Harkins. Both hypotheses are ingenious interpretations of a little understood and difficult problem.

The solubility theory of Bancroft is based on the difference in the surface tension of the two sides of the stabilization membrane. If the emulsifier is soap, and we imagine it to be a flat membrane with water on the one (left) side and oil on the other (right) (Fig. 84), then the way it will bend, and therefore whether it will enclose water or oil, is determined by the difference in the tensions of its two surfaces. That side with the higher tension

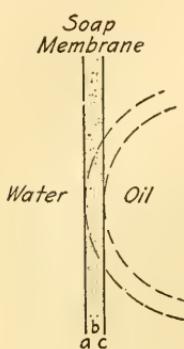
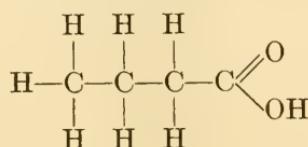


FIG. 84.—Diagram of a soap membrane (*b*) with its two interfacial films (*a* and *c*) the relative tensions of which, due to relative solubility in oil and water, will determine the direction of bend of the membrane and therefore the type of emulsion.

will force the membrane to bend its way; which side this will be is determined by the degree of solubility of the membrane substance in oil and in water. For example, alcohol and water mix fully and freely; consequently, there can be no membrane between them; oil and water do not mix at all, and the membrane separating them is at high tension. Imagine that the particular kind of soap forming the membrane around an emulsion droplet mixes more readily with water than with oil; then the water side of the membrane will be at a lesser tension than the oil side. This being the case, the soap-oil interface, having the higher tension, will bend the stabilization membrane toward the oil in opposition to the weaker pull of the soap-water interface (Fig. 84); an oil-in-water emulsion is the result. If we know that a sodium soap is more soluble in water than in oil, then we can predict that it will give an oil-in-water emulsion, which it does; and if a calcium soap is more soluble in oil than in water, then it should give a water-in-oil emulsion, as it does. This explanation tells also why a calcium salt added to an oil-in-water emulsion stabilized with a sodium soap will reverse the emulsion to the water-in-oil type.

The molecular-wedge hypothesis of Harkins and Hildebrand had its origin in the work of the physical chemist Irving Langmuir. In a most ingenious manner Langmuir was able to make oil molecules stand up, lie down, or lean at an angle when on water and to know which position they were in. He used a very shallow, rectangular vessel containing water on which was spread a thin (monomolecular) film of oil. A bar, just touching the water surface lightly, was freely suspended above so that

when the oil film thickened or thinned, the bar moved, and the motion was recorded by levers and a scale. The substances with which Langmuir worked were the higher fatty acids, the molecules of which are long chains. The simpler ones in the series, such as formic and acetic acids, are water-soluble and therefore do not form oily films. The higher ones, such as stearic acid and the closely related oleic acid, resemble oils and are insoluble in water. Their molecules are long, with a carboxyl group at one end. The structural formula of one of the simpler of these fatty acids, butyric ($\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$), is



It is evident that the molecule is polar, *i.e.*, its two ends are different, and it is further known that the carboxyl (COOH) group at the active polar end is more readily soluble in water, because of its OH radical, than is the oily organic CH_3 radical at the other end. In the case of higher fatty acids, the chain is much longer, as in oleic acid, with its 17 carbon atoms ($\text{C}_{17}\text{H}_{33}\text{COOH}$). Such linear molecules, with one, the COOH , end water-soluble and the other, CH_3 , end not, will very naturally stand on end when on the surface of water. The union between the carboxyl end of the chain molecule and the water is one of residual valence, which is merely the chemist's way of saying that he knows only that the bond is weaker than a primary-valence one. The molecules of such organic substances when at the interface between oil and water, as in emulsions, will orient themselves with the COOH ends protruding into the water on the one side and the CH_3 ends protruding into the oil on the other. With these facts in mind, Hildebrand considered the case of soap films as stabilizers of emulsions.

Soap molecules are polar. In a soap, such as sodium oleate, the polar end is the metal sodium, and the nonpolar end the organic radical. Such molecules will orient at the interface between oil and water so that the metal end is in the water and the organic end in the oil (Fig. 85). If the molecules are of the same size at both ends, they will, when packed together in a row, assemble in a straight line; but if the ends are not alike, *i.e.*, if

the molecule is shaped like a wedge, then the row will bend (Fig. 86). The way it bends will depend on which end of the soap molecule is the larger. In the case of a sodium soap, it is the metal end, of sodium, protruding into the water, which is the larger, and the organic end, protruding into the oil, which is the

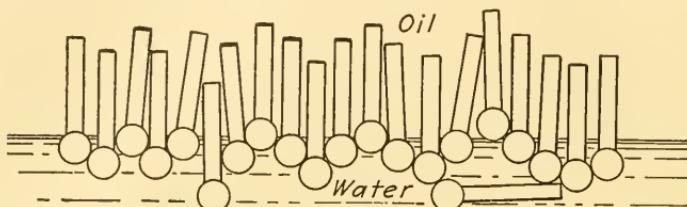


FIG. 85.—Polar (stearic acid) molecules oriented at an oil (benzol) water interface.

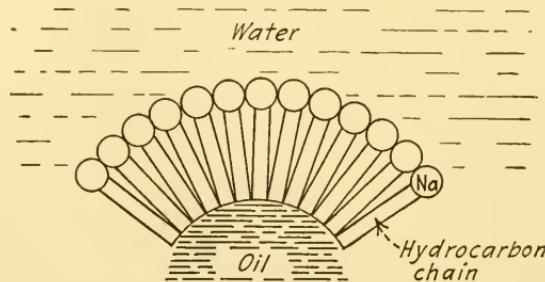


FIG. 86.—Sodium stearate (soap) molecules at the interface of oil globules in an oil-in-water emulsion.

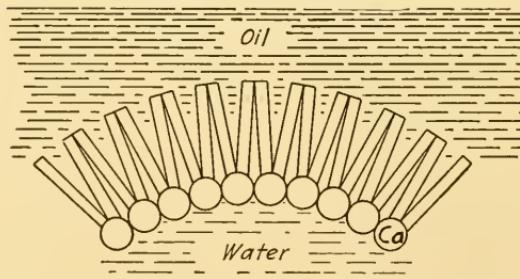


FIG. 87.—Calcium stearate (soap) molecules forming the stabilization membrane of a water-in-oil emulsion.

smaller. Such a membrane will curve so that the larger sodium ends form the outer surface. This will be on the water side, and the membrane will surround oil on the inner side. The resulting emulsion is, therefore, an oil-in-water one (Fig. 86). Theory demands and experiment proves that a sodium soap forms an oil-in-water emulsion.

If the metal of a soap has a higher valence than one and holds more than one hydrocarbon chain, the oil end of the soap molecule will be larger than the metal end, and the membrane will bend in the reverse direction; the emulsion then becomes a water-in-oil one (Fig. 87). Again, theory demands and experiment proves that calcium soaps, with a bivalent metal, form water-in-oil emulsions. If this hypothesis holds generally, then all soaps of monovalent cations, such as sodium, potassium, silver, and caesium, should form oil-in-water emulsions, and all soaps of bivalent cations, such as calcium, magnesium, and zinc, should form water-in-oil emulsions, and this they do.

Hildebrand carried this theory further and gave it very convincing experimental support. He argued that soaps with trivalent cations should produce water-in-oil emulsions as do bivalent ones but that these emulsions should have smaller droplets and therefore should be more stable systems, because three hydrocarbon chains on one metal atom will give a broader wedge and therefore a sharper curve, a smaller globule, and a more stable emulsion. Furthermore, in any one group, of mono-, di-, or trivalent metals, the stability of the emulsion should be determined by the size of the metal atom, because it determines the slope of the wedge. It would seem, therefore, that the direction and degree of curvature of the membrane and therefore the type and stability of the emulsion are determined by the size and the valence of the metal. The following table¹ substantiates this:

Metal	Valence	Atomic volume	Type of emulsion	Life of emulsion
Na.....	1	22.9	O W	6 weeks
K.....	1	45.3	O W	8 weeks
Cs.....	1	70.6	O W	8 weeks
Ca.....	2	12.6	W O	1 hour
Mg.....	2	7.0	W O	2 days
Zn.....	2	4.6	W O	24 days
Al.....	3	3.4	W O	7 days
Fe.....	3	2.3	W O	10 days

The theory is but weakly supported by the monovalent metals, although affirmatively so, for the large metal caesium forms a

¹ Data from Hildebrand.

more stable oil-in-water (O W) emulsion than the smaller metal Na. The theory is better supported by the divalent metals. In water-in-oil (W O) emulsions, where the metal forms the inside of the curve, the smaller the metal the sharper are the wedge and the bend, the smaller the (water) globule, and the more stable the emulsion. The larger metal calcium forms a poor wedge and an unstable emulsion; the smaller magnesium forms a finer and more stable emulsion; and zinc, the smallest of the three, yields the best emulsion because it forms the sharpest wedge.

The oriented-wedge hypothesis has much in its favor, but stabilization membranes are not always just one molecule thick, and there is no reason to believe that the molecules are actually and sufficiently wedged to make a sharp curve, nor can we be certain that the metal end of the wedge will "stay put"; it may ionize. The English chemist Clayton, who has brought together all-important work on emulsions, warns us not to become overenthusiastic about monomolecular films. There is experimental evidence to indicate the presence of films many molecules thick on emulsion globules. Saponin when used as a stabilizer may form films 40μ thick. The irregular shape of oil globules, so often seen in artificial and natural emulsions, is due to plastic films of colloidal dimensions. The globules of latex are pear-shaped, their form being maintained by a stiff membrane (Fig. 89). Blood corpuscles are surrounded by a delicate but resistant membrane which encloses the liquid hemoglobin. In spite of a nonrigid content which should give the droplet a spherical shape, the red blood cell of Amphibia is a flattened disk, and of human beings an invaginated disk. The shape is maintained by the rigidity of the membrane.

Membranes, when first formed in an experimental emulsion, are too delicate to be seen microscopically, but often, especially when electrolytes have been added, they thicken and stiffen and may, when the emulsion breaks, separate from the globule and maintain their identity like so much crumpled tin foil.

Solids as Emulsifying Agents.—Solid matter tends to enter a liquid that wets it and to remain at the surface of one that does not wet it. If particles of clay, which are more readily wet by water than by oil, are shaken with oil and water, they will enter the water and line up at the surface of the oil. The result is an

oil-in-water emulsion (Fig. 88). Colloidal clay (kaolin) and lampblack make good emulsifiers. Many colloidal suspensions and precipitates (ferric hydroxide and basic copper sulphate) stabilize emulsions well.

Certain Arab tribes, and in general primitive people the world over, use fine earth for washing. This method of cleansing and our more civilized use of soap involve identical methods, *viz.*, emulsification. Both fine earth particles and soap emulsify the film of oil on our hands and thus permit washing the oil away. An oil-in-water emulsion leaves a surface wet with water and therefore relatively clean.

Breaking of Emulsions.—When an emulsion is being reversed with an electrolyte, there is a theoretical point at which no emulsion exists. In practice, before the last vestige of one type has disappeared, a small quantity of the other type is already formed; but while there is usually no sharply marked *breaking point*, there is always a region in which the emulsification is very poor. Emulsions may be broken by adding just enough of the proper electrolyte to de-emulsify. Breaking an emulsion by gradually bringing it to the reversal point with electrolytes is not what the commercial chemist usually has in mind when he speaks of breaking. He is thinking of emulsions that were good when first formed but later break or separate. Mechanical agitation such as jarring may cause an otherwise very stable emulsion to break. Thomas cites a number of interesting cases of the breaking of emulsions by agitation. Vibrations from shipment by truck or railway may incite breakdown. This is known in commercial mayonnaise handling, where trucks equipped with special springs to reduce shock are sometimes used for the transportation of mayonnaise. The reverse situation exists in the finely emulsified water in petroleum distillates. The emulsion is so stable that getting the water out presents a very difficult commercial problem. On the other hand, an oil-field emulsion, so stable as to resist separation in a centrifuge, separated into water and oil during transit in an express train. Once an emulsion breaks, it is usually re-emulsified with difficulty.

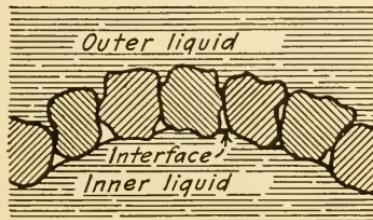


FIG. 88.—Solid particles at the surface of an emulsion globule functioning as a stabilization membrane.

Emulsions possess many extraordinary peculiarities. Among them is the effect of resting on the type of the emulsion. One-half of a sample left on the laboratory table, while the other half is being used, may reverse to the opposite type on shaking fifteen minutes later, owing simply to changes going on while resting. Remarkable also is the effect of rest on the quickness with which emulsions are formed. Intermittent shaking produces an emulsion much more rapidly than does constant shaking. When forming, emulsions are especially sensitive. The method of preparation has a pronounced effect on the success of the emulsification and also on the type. Any housewife of a generation ago will testify to this on the basis of her attempts to make mayonnaise. The mayonnaise emulsion problem is commercially now well in hand, but the reasons for the success are little understood.

No system, except the living substance itself, exhibits more idiosyncrasies than do emulsions, and for most of these we have no adequate explanation.

Supersonic Waves.—A novel way of forming emulsions has arisen as a result of studies by the French physicist Langevin (1923) on sound waves of exceedingly short length—so short as to be below audibility by the human ear. They are known as *ultraphonic*, *ultrasonic*, or *supersonic* waves. They are produced by the vibration of a quartz crystal (plate), brought about by subjecting the crystal to an alternating current of high frequency, the wires of which are wrapped about the crystal. The crystal trembles, owing to the electrical oscillations to which it is subjected. This trembling brings about the production of waves in the medium in which the quartz is suspended; the medium usually employed is a neutral oil. The vibrations are purely mechanical, like those of a tuning fork but much shorter. The range in audibility of sound waves for the human ear is in air from 200 m. to 16 mm. Supersonic waves are all below 16 mm., generally 2 or 3 mm. Variations in the frequency of the current cause the production of waves of different length.

If a coarse mixture of oil and water is subjected to supersonic vibrations, a very fine emulsion is produced in short time. If a gel that exhibits thixotropic qualities (page 150) is set in vibration by supersonic waves, it will collapse just as it does when mechanically agitated by more crude disturbances.

The effects of supersonic waves extend into the living world; bacteria and other one-celled organisms are killed by them. This fact has led to a consideration of the method as a means for the sterilization of milk. Bacteria in milk would presumably be killed without the milk acquiring the taste that heating gives to it. Still another change is produced in the milk which is also of advantage: the butterfat is more highly emulsified. The droplets, now being smaller, are more readily digested. The process is in the experimental stage as yet but has commercial possibilities.

Natural Emulsions.—It is quite obvious that wherever oil, fatty, or other liquid substances insoluble in water occur in nature, there is the likelihood of their being finely dispersed in their aqueous medium. Such natural emulsions are found in both the nonliving world (as in petroleum) and the living world. Among the latter is latex, the rubber-producing liquid occurring in a large variety of plants (*Ficus elastica*, *Hevea brasiliensis*, *Euphorbia*, *Solidago*, etc.). On coagulating, latex forms a tough elastic mass which is crude rubber. The latex emulsion consists of dispersed globules of a hydrocarbon oil, stabilized by protein and resinous matter. Hauser has studied the latex globule by microdissection methods and finds it to consist of at least four layers (Fig. 89) three of which are of hydrocarbons which increase in polymerization from the inner, less viscous, ether-soluble core to the outer, more viscous gel which is insoluble in ether. It was formerly thought, and may yet be true in part if not in whole, that when latex coagulates it is the protein covering of the oil globules that coagulates, causing the globules to adhere, thus forming a continuous, spongelike structure the elastic qualities of which reside in the protein coagulum and not in the oil emulsion as such. (This would be true of a protein stabilized emulsion of any inelastic oil, such as those met with in daily life.) But now it is believed that the elastic properties of rubber reside in the oil itself, owing to the unique structure of the hydrocarbon

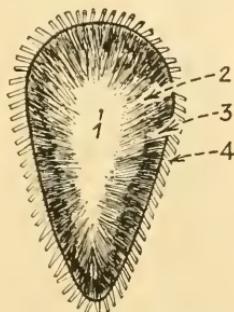


FIG. 89.—The latex (rubber) globule with (1) the inner kernel of low weight hydrocarbons; (2) intermediate layer of increasing polymerization; (3) outer hydrocarbon membrane of maximum polymerization; (4) adsorbed layer of resin and protein material. (From E. A. Hauser.)

molecule which is presumed to be an isoprene, linear in form and spirally wound (a helical coil). That this is true seems likely from the successful attempts (by W. H. Carothers of the du Pont Laboratories) to produce artificial rubber. Synthetic rubber is an emulsified isoprene (α -polychloroprene) which is not stabilized with protein membranes; the elastic qualities cannot therefore reside in the latter.

The Protoplasmic Emulsion.—Among natural emulsions, that which is of most interest to us is the protoplasmic one. The behavior and significance of the living emulsion we shall have occasion to refer to very often. In its coarser, microscopically visible structure, protoplasm is an emulsion of fat, yolk, and other globules dispersed in a complex aqueous medium; but this structure is a superficial one and not of fundamental significance. Those who regard protoplasm as a fine emulsion in its ultramicroscopic structure have developed some interesting theories based on this assumption. These will be taken up under the vital processes that they are said to explain.

CHAPTER VIII

HYDROPHILIC SOLS AND GELS

The aqueous medium of protoplasm contains salts and carbohydrates in true solution (molecular dispersion). The visible structure of protoplasm is that of an emulsion. But neither of these systems constitutes the ultimate substratum of life processes. Those properties of protoplasm which characterize it fundamentally, such as imbibition, elasticity, and coagulation, are properties of jellies. The jelly-like nature of protoplasm gives to it that continuity in structure, or organization, so necessary as a background for the multiplicity of reactions which, combined, constitute life. It is, therefore, obvious why a knowledge of gel-forming systems is so important to the study of protoplasm.

Types of Gels.—The hydrophilic colloidal systems, of which gelatin is an example, are characterized by their capacity to form *gels*. The liquid state of these systems is known as a *sol*. Graham took the first three letters of *gelatin* and *solution* to indicate the firm and the fluid conditions of colloidal substances. When a solution of gelatin is hot, it is a *sol*; on cooling (if sufficiently concentrated), it *sets* into a *gel*. Some of the properties of gels are retained in their sols; thus, firm gelatin jelly is elastic, and so also is liquid gelatin. It is their gel qualities that so thoroughly distinguish the hydrophilic colloids.

Special terms have been devised to designate sols and gels on the basis of the nature of their dispersion medium, *e.g.*, *hydrogel*, *alcosol* and *xerogel*. The last name was coined by Freundlich to indicate a dry gel such as sheet glue. When sols become firm, they are said to *gelate*. The reverse process is *solute*. Gels that swell when they take up water are known as *jellies*; they are also elastic. Sheet gelatin and glue form jellies when soaked in water. Jellies, because they swell, are said to be *turgescent*. Gels that do not swell in water are called *coagula*; they are not usually elastic in the sense of being extensile (rubber is an exception). When blood becomes firm on exposure to air, when milk turns into clabber or cheese, and when albumin is heated,

coagulation takes place, and coagula are formed. Certain coagula have special names, *e.g.*, a soap *curd*. Another property which is rather generally characteristic of jellies but not necessarily true of coagula is *reversibility*. Firm, hydrated gelatin can be made liquid simply by heating, and the sol thus formed will again set to a gel. Such simple reversibility does not take place in true coagula. Heated albumin forms an irreversible coagulum.

The divergent meanings that have been given the term gel and related expressions are best harmonized by defining gel so that it will include *all* types of firm lyophilic (and some lyophobic) colloidal systems, with jellies and coagula as subtypes. In this way will these terms be used on the following pages. Jellies, then, are reversible gels; they set by stiffening, by the process of gelatinization; they are elastic and swell in water. Coagula are (strictly) irreversible gels; they become firm by the process of coagulation; they are usually relatively inelastic (*i.e.*, of very low extensibility), and they take up but do not swell in water.

There are a number of other terms which describe processes similar to if not identical with gelation or coagulation. These are *precipitation*, *agglutination*, *agglomeration*, and *salting out*. Such terms were first used when nothing was known of the mechanism of the processes; the end products simply looked different, or the original substances were of a different kind. Different names were therefore given to the processes; thus, blood coagulates, salts and colloidal suspensions precipitate (though coagulate is also used here), and bacteria agglutinate. The result in each case may be the same—a clumping together of particles into aggregates which cannot usually be readily redispersed. Various attempts have been made to restrict these terms (*e.g.*, coagulate to organic systems such as blood and milk), but no success has come of it, primarily because the more that is known about them the more does it appear that they are fundamentally alike. We may, however, distinguish between the gelatinization of a reversible system (gelatin) and the coagulation of an irreversible one (albumin).

Attempts have been made arbitrarily to state when a gel ceases to be a gel and becomes a sol. All such attempts lead to confusion. One can say that a gel firm enough to maintain its shape unsupported is a true gel but if it collapses, it is a sol. However, this is purely arbitrary, and a collapsed gel is not properly

termed a sol unless it flows freely. More important is it to realize that one of the distinguishing features of organic (lyophilic) colloidal systems is their capacity to exist in a state that is not truly solid or truly liquid and to carry some of the characteristic properties of the one state over to the other.

Methods of Preparation.—A gelatin gel is easily prepared by allowing dry gelatin to swell in water or by dissolving gelatin in hot water (3 grams or more in 100 cc. of water) and allowing the solution to cool. Dry sheet gelatin swells to many times its original volume when put in cold water, but it does not go into solution, *i.e.*, dissolve, until heated above 30°C. A poor grade of glue goes into solution in cold water. Similar gels may be prepared from agar (agar-agar) which is very like gelatin in its behavior, though quite a different substance chemically. Gelatin is a protein from animals, and agar a carbohydrate from plants. It is agar that causes some seaweeds (*dulse* and "Irish moss") to set to jellies when prepared for desserts. A 1 per cent solution of agar in hot water sets to a firm jelly when cold. Other naturally occurring jellies are egg white and vegetable gums (the former is a fluid gel, which means that it possesses the capacity to flow yet has other gel qualities, such as elasticity).

The most familiar example of a coagulum, the irreversible type of gel, is the clot formed by blood. Differing from it in some respects, yet a typical coagulum, is the silicic acid gel. It is made by adding moderately strong hydrochloric acid to an equal volume of sodium silicate (waterglass) of about 1.15 sp. gr. Free silicic acid, H_2SiO_3 , is formed, which, being insoluble, is precipitated as a gelatinous coagulum; this sets to a firm gel of a slight bluish opalescence. The time of setting may be instantaneous or prolonged to a day or more, depending on the concentration of acid and silicate used. When freshly made, the gel is of silicic acid; but when dehydrated, it becomes pure silica (sand). The chemistry of the process is $Na_2SiO_3 + 2HCl = NaCl + H_2SiO_3 \rightarrow SiO_2 + H_2O$. The hydrated gel is springy but not extensible. Dry silica gel is as hard as glass. It takes up water and other substances but is nonturgescible. Among the extraordinary properties of some gels is one especially well exhibited by the hydrated silica gel. When given a sharp blow, the gel will ring. If the concentration and the form of the gel are suitable, the sound is musically pleasant.

Lyophobic Gels.—We have so far considered two main types of gels—the organic lyophilic ones which take up water, swell, and form elastic jellies, such as gelatin, albumin, agar, vegetable gums, and cellulose; and the inorganic lyophilic ones, such as the silica gel, which also take up water but do not swell and are not extensible. There are exceptions like rubber, which is of organic material and highly elastic but not lyophilic; *i.e.*, it does not take up water. There is still another class of gels—those of the oxides or hydroxides of metals. These gels are of inorganic material and lyophobic; they do not absorb water. The fact that they are oxides of metals tends to put them with the suspension colloids, but their gel-forming capacities class them as lyophilic colloidal systems; they are thus border-line systems.

Metallic oxides that form gels are Fe_2O_3 , CuO , V_2O_5 , Al_2O_3 , Se_2O_3 , ZrO_2 , and SnO_2 . Best known among these lyophobic inorganic gels is that of iron oxide. When crystals of ferric chloride are thrown into hot water, an insoluble and colloidally dispersed oxide or hydroxide of iron is formed. Such a suspension is indiscriminately called an oxide or hydroxide, because it is not known with certainty whether the colloidal particles in suspension are $\text{Fe}(\text{OH})_3$, Fe_2O_3 (FeOCl)_z, or some other form. The vanadium pentoxide, V_2O_5 , sol will probably always go by that name even though the particles may be a hydroxide. Boehm studied the iron oxide sol and believed it to be mostly of basic iron chloride, $\text{Fe}(\text{OH})\text{Cl}_2$, but also in part hydrated iron oxide, $\text{FeO}(\text{OH})$. This can be interpreted to mean that the core of the colloidal particle is iron oxide, and the surface a shell of adsorbed water and HCl. Heller added to and supported this viewpoint but with emphasis on the hydroxide ("Goethit," one of the most common hydroxides of iron in nature), with the basic salt as incidental. It seems probable that most of the metallic oxide solutions are hydrated. Choosing ferric hydroxide as representative of the group, the metallic colloidal hydroxides must, if not classed separately, be put with the lyophobic systems, not only because the precipitated basic salt shows little affinity for water but also, and perhaps primarily, because the sol is very sensitive to electrolytes.

Imbibition.—The taking up of water when accompanied by swelling is *imbibition* (Chap. XII); the resulting condition is known as *turgescence*. Imbibition is characteristic of jellies but

not of coagula. The pressure thereby exerted may be tremendous. Peas packed into a bottle, then covered with water and tightly corked break the bottle on swelling. The swelling of wood may split rock, as done by roots swelling in rock crevices and artificially by wooden wedges used by the ancient Egyptians for quarrying stone. Starch in water may exert an imbibition pressure of over 2,000 atmospheres (the pressure in the average locomotive boiler is 10 atmospheres). Gels on shrinking also exert a pull. Dehydrating (drying) gelatin pulls with sufficient force to chip the glass to which it is attached.

Heat of imbibition, or heat of swelling, or heat of wetting holds the same important position in the thermal chemistry of colloids and the theory of colloidal systems as do heat effects in ionic systems when these go into solutions. Every freshman in chemistry knows how hot a beaker of water will become when sulphuric acid is added to it. Swelling jellies get hot in the same way, though not so much so. Heat of imbibition is expressed in calories per gram of substance. It may give rise to an increase of 0.2 to 0.9°C. when carbon, starch, cotton, or animal membranes are wet.

Heat of wetting has been used by Bouyoucos to define soil colloids. The question whether the colloidal state shall be distinguished on the basis of structure (size of particle) or of activity (energy manifestations), such as heat of imbibition, is here answered in favor of the latter dynamic viewpoint. Again we can say, we inquire of colloids not what they are but what they do. The inevitable difficulty arises, as in the case of all criteria of the colloidal state, that there is no sharp line of demarcation. Heat of swelling is not wholly present or wholly absent. Its apparent presence or absence depends on the sensitiveness of our method for measuring it. However, it can be said that the greater the dispersion (*i.e.*, the smaller the particles) in soil the greater is the heat of wetting. To this extent is heat of imbibition a measure of the colloidal state in general.

Adsorption.—Adsorption (Chap. X) is the concentration of one substance at the surface of another.

Silica gel, when thoroughly dry, is hard, glassy, and very finely porous (Fig. 90). It functions as an ideal adsorbent of many things. Formerly, charcoal played this role in the commercial world, but through the development of better methods

in preparation, silica gel has now attained wide commercial use in America as an adsorbent, for drying blast-furnace air, decolorizing solutions, deodorizing refrigeration air, and purifying air for breathing by adsorbing poisonous and obnoxious gases. The efficiency of silica gel as an adsorbent is due to its great number of, and exceedingly minute, pores ($5\text{ m}\mu$), which offer a tremendous surface for adsorption. In other countries, "active" charcoal is still the preferred adsorbent.

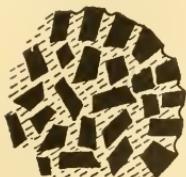


FIG. 90.—Porous structure of a silica gel.

into a glass of water becomes uniformly distributed throughout the water. This movement of gases and dissolved substances from a region of high to one of low concentration is termed *diffusion*. Ions migrate at different speeds, hydrogen being the fastest. The rate of migration or diffusion of a molecule, atom, or ion is in part determined by its size; therefore hydrogen, being the smallest, should and does travel the fastest. Calcium and chlorine travel at about the same rate; they are separated in the periodic table by but one element, which indicates that they are very nearly the same size. Lithium, on the other hand, is separated by but one element from hydrogen, yet it moves much more slowly. The greater speed of hydrogen over that of any other element is probably due to the unique condition of this atom when ionized, for then it is stripped of its lone negative electron, and there remains but a single proton or positive charge, unhampered by outer electrons. The much slower diffusion rate of the nearby lithium atom is probably also due to the fact that it is highly hydrated. Size, mass, temperature, and viscosity of the medium are determining factors in the rate of diffusion of atoms, but hydration may dominate over these. Thus, the order of diffusion rate of three elements is $\text{Li} < \text{Na} < \text{K}$, while the order of size is the reverse; furthermore, chlorine, bromine, and iodine have the same diffusion rate in spite of considerable difference in size.

Size and mass probably play the chief part in determining the rate of movement of the mammoth-sized molecules of proteins, although here, again, hydration is a prominent factor. Egg

albumin with a molecular weight of 34,000 (hydrogen being 1) diffuses very slowly. Whether or not it is the molecules or the micelles (molecular aggregates) of proteins that diffuse in solution, it is impossible to say with certainty, but whichever they are, they are colloidal in behavior. The diffusion rate of all colloidal particles is very low.

The following diffusion constants are of several colloidal and crystalloidal substances:

DIFFUSION CONSTANTS

($D = \text{cm.}^2/\text{sec.} \times 10^5$)

Egg albumin.....	0.063	Urea.....	1.01
Pepsin.....	0.073	Sodium ion.....	4.51
Glucose.....	0.57	Hydrogen ion.....	32.50

Albumin, colloidal in nature and with a molecular weight of 34,000, moves slowly; the sugar glucose, which is crystalloidal, with a molecular weight of 180, moves much faster; urea, with a molecular weight of 60, is still faster; sodium, of atomic weight 23, is faster yet; and hydrogen, with a weight of 1, is fastest.

If rate of diffusion is in part dependent upon size and mass, it should be possible to calculate one of the latter two, if the other and the diffusion rate are known. This can be done with the aid of two formulas derived by Einstein. The radius of egg albumin so determined is $2.8 \text{ m}\mu$.

Diffusion studies in colloids concern not only the movement of the colloidal substance itself (albumin in water) but also those of other substances through the colloids (salt through gelatin). Graham suspected that substances diffuse more slowly through gels than in free liquids, but he found common salt to diffuse in a gelatin gel just as quickly as in pure water. His surmise and his experiment were both correct, as many substances diffuse far more slowly in gels of high concentration (10 per cent or more) than in water, but in dilute gels the diffusion rate of a substance is approximately the same as in a free liquid. The rate decreases rapidly with increase in concentration of the gel.

Retardation in diffusion rate through gels may be caused by adsorption forces operating between the diffusing substance and the inner surfaces of the gel, but it is probably the purely mechanical features (structure) of the gel that are primarily responsible. If the structural units are close together, as in a highly concen-

trated gel, the diffusing particles will have more difficulty in getting through; but if the gel is open in structure, then there will be little hindrance to the diffusing substance.

Ruhland has characterized the aqueous solutions of a large number of dyes by determining their rate of diffusion in a concentrated gel (20 per cent gelatin). One can thus ascertain with moderate accuracy whether the diffusing substance is molecularly or colloidally dissolved, *i.e.*, whether the particles are molecules or micelles.

Osmosis.—*Osmotic pressure* (Chap. XI) is a measure of the pressure that a solution would exert were it confined in a sack under certain ideal conditions. It was named by Graham as one of the forces not possessed by colloidal solutions. When a sugar solution is enclosed in a sack of parchment paper immersed in water, a hydrostatic pressure is set up within the sack owing to the entrance of water. The entering water is in excess of the outgoing water, because of the inability of the sugar to diffuse out. The greater the number of sugar molecules the greater is the difference between the incoming and outgoing water, and therefore the greater the hydrostatic pressure developed within. Protein molecules and colloidal particles are large. They occupy space, and there are fewer of them per gram of substance; consequently, in equimolecular concentrations of crystalloids and colloids, there are fewer of the latter per unit volume in the osmotic sack, which means that the difference between incoming and outgoing water, and therefore the hydrostatic pressure, will be less. Colloidal substances do, however, possess a definite though slight osmotic pressure. Pfeffer measured it for gum and glue solutions. A colloidal substance with a molecular weight of 20,000 will give an osmotic pressure of 7 mm. of mercury—a low value compared to a crystalloid, such as cane sugar, with a molecular weight of 342, which will yield a pressure of 25.69 atmospheres, or 1,850 mm. of mercury, at a concentration of 0.1 *M*. The amount of osmotic pressure exerted by colloidal solutions is so slight that it is customary to regard its absence as characteristic of the colloidal state.

Size of Particles in Hydrophilic Sols.—The slow diffusion rate and the almost negligible osmotic pressure exerted by hydrophilic colloidal systems are due to the large size of the dispersed particles. High molecular weight suggests that the “particles”

in the case of proteins are molecules, though they may be micelles or aggregates of molecules. Around this question have centered the most severe of the controversies in colloid chemistry. The concept of a colloidal particle, a micelle, as the unit of structure in colloidal systems, has dominated the thoughts of most colloid chemists. That such particles exist in lyophobic systems (*e.g.*, colloidal gold) there can be no doubt, but the term micelle is applied primarily to the structural unit of lyophilic systems (*i.e.*, gelatin). An attempt at a solution of this problem in so far as it applies to gel structure will be reserved for a later chapter. For our present purpose (*i.e.*, as an explanation of slow diffusion rate and low osmotic pressure), it is sufficient that the particle is large, whether micelle or molecule, and this condition is met by the very large size of the protein molecule. This does not preclude the possibility of these molecules being grouped together to form colloidal aggregates.

Little is known of the nature of the structural units of jellies. Coagula are in general granular as is true of the silica gel. Here we undoubtedly have to do with a very finely porous framework built up of colloidal granules (Fig. 90). In the case of the sols and gels of metallic oxides, there is also little doubt but that typical colloidal particies are the structural units.

Hysteresis.—*Hysteresis*, a word of Greek origin meaning a “deficiency” or a “coming after,” is applied to two quite distinct physical phenomena, one in electrical engineering and one in colloid chemistry, which have, however, one thing in common, *viz.*, a lagging of one process behind another. When gelatin is repeatedly melted and allowed to resolidify, the temperature of the melting point and also of the solidifying point becomes progressively lower. This behavior is known as hysteresis. It is an expression of the past history of a substance, and upon it do a number of properties, such as hydration and viscosity, depend.

An extraordinary case of hysteresis is the effect on a gel of the degree of previous swelling. A 10 per cent gelatin jelly will, if dried to 97 per cent gelatin and allowed to swell again in water, swell to a 10 per cent gel when it otherwise would have taken up much more water. A 30 per cent gelatin gel under the same conditions swells again to approximately a 30 per cent gel. We see, therefore, that the present behavior of a colloidal system

is determined to a great extent by what it has previously experienced.

The setting temperature of a gel is always a few degrees lower than the point of liquefaction. As a result, there exists a hysteresis range within which the colloidal system may exist either as a sol or as a gel. This range is much greater with agar than with gelatin. Firm, hydrated agar does not liquefy until heated to 95 to 100°C. but does not set to a gel until cooled below 35°C., and this gel must again be heated to about 95° to be converted into a sol. There is thus a range of 60° within which the system may exist as either sol or gel. For gelatin, the hysteresis range is but 5 to 10°C.

Syneresis.—*Syneresis*, a word meaning a “drawing together,” is the contraction of gels which results in the giving off of water. The gel “sweats.” The process is best observed in a mixture of 2 per cent rubber and 2 per cent sulphuric chloride in benzol; equal volumes of the two solutions rapidly mixed will soon set to a gel which shows syneresis in about twenty minutes.

The separations of serum from clotted blood and of sour milk into curd and whey are syneresis phenomena. The housewife has to deal with syneresis in the making of foods that involve the setting of albumin, gelatin, or other protein into a jelly, which later, contrary to the housewife’s plans, gives off some of the water it holds, *i.e.*, separates, sweats, or leaks by syneresis.

The exudation of water and other secretions from protoplasm and tissues (glands) may, in certain instances, be syneresis.

Stability.—The stability of lyophilic and lyophobic colloidal systems (gelatin and gold suspensions) is, in both instances, due to the immediate environment of the particle, but the nature of the stabilizing envelope may differ in the two cases. Aqueous suspensions of gel-forming substances such as gelatin, casein, gum, and soap probably stay up because of adsorbed water rather than adsorbed ions, though the distinction may not be a very great one, in that we may have to do with electrical forces in both cases. The water molecule is polar (Fig. 138), which means that it is electrically unlike at its two ends. It will, consequently, be attracted (adsorbed) and held by a charged protein molecule or colloidal particle just as in the case of an ion. Water dipoles will orient themselves around protein molecules like a lot of magnets, with one end or the other facing inward, depending on

the sign of the protein particle (Fig. 177). The adsorption of water in this manner is known as *solvation*. The Dutch chemists Kruyt and de Jong believe that proteins and lyophilic colloids in general are kept in suspension by both adsorbed water and adsorbed ions. When more water molecules than ions surround the protein particle, it is less sensitive to electrolytes (salts) and is precipitated best by "desolvating" agents such as alcohol. When less water is present, the system is more sensitive to electrolytes. It is generally true that solutions of lyophilic organic matter are less susceptible to the effects of electrolytes than are suspensions of metals. Freundlich believes these electrical properties to be a natural basis for classification and has called the lyophobic colloids *electrocratic* (electrosensitive) in contrast to the electroresistant lyophilic ones.

An adsorbed envelope of ions or a water mantle or both are apparently responsible for the stability of lyophilic colloidal systems (gelatin, albumin, and soap). When both ions and water stabilize, precipitation is accomplished only by a substantial reduction in both charge (removal of the ionic envelope) and hydration (removal of the water mantle).

Gortner says that the salting out of lyophilic colloids (a process common in the manufacture of soap) by the addition of ammonium sulphate is not an electrokinetic phenomenon, whereby the electrical charge of the protein in solution is reduced to zero, but a dehydration of electrically neutral micelles.

Protective Colloids.—The greater resistance of the lyophilic colloidal systems (gelatin) to electrolytes can be transferred to the lyophobes (gold) by giving the solid particles of the latter a coating of the former. Most organic colloids, such as gelatin and albumin, will function in this way and are known as *protective colloids*. Gelatin when added in hot solution to a colloidal dispersion of a metal forms a thin membrane around each metal particle and thus protects it from the action of an electrolyte. (Organic substances function as protective colloids only when in proper concentration. They may, when in slight concentration, actually make a lyophobic colloidal suspension more sensitive to electrolytes.) Zsigmondy has made a special study of the protective efficiency of organic substances and has given a value to each, the so-called *gold number*, which indicates their relative protective powers. The gold number is that weight in milli-

grams of the lyophilic colloid (gelatin) which just fails to prevent a change in color from red to violet when 1 cc. of a 10 per cent solution of sodium chloride is added to 10 cc. of Zsigmondy's (formaldehyde) red colloidal gold. Some gold numbers are

Gelatin.....	0.005 to 0.01	Dextrin.....	10 to 20
Egg albumen.....	0.06 to 0.30	Potato starch.....	25

Gelatin is the most efficient among the protective colloids; *i.e.*, less of it is needed, because it is the most resistant to electrolytes.

Findlay has given some examples of the protective action of substances. He tells us that the waters of the Mississippi and of the Nile are always muddy owing to the presence of large amounts of colloidal organic matter which stabilizes the fine suspension of clay. The water of the Ohio river, on the other hand, is clear owing to the absence of protective colloids and the presence of lime and other salts which act as precipitating agents.

The protective action of organic matter plays an important part in physiological processes. Findlay cites the case of bile albuminoids which act as protective colloids and keep poorly soluble substances, such as cholesterol and the salt of bilirubin, in the colloidal state and thus prevent their deposition as gallstones until pathological conditions interfere.

When milk curdles, it is the casein which does so. The process is made less easy by the presence of lactalbumin in milk, which is more resistant than is casein to the electrolytes of the stomach and therefore partially protects the casein from coagulation. Cow's milk is less easily digested than human milk, which is less digestible than ass's milk. The reason probably lies in the relative proportions of casein and lactalbumin in the three cases, there being least lactalbumin in proportion to casein in cow's milk, and most in ass's milk. Proof of the theory lies in the fact that the digestibility of cow's milk can be increased by adding gelatin, white of egg, or barley. Curdling then occurs less readily, owing to increased protection by these colloids.

The gold number finds application in medicine in the diagnosis of certain diseases. The gold number of the spinal fluid of a normal person is a definite value; any deviation from it is indicative of disease. Certain afflictions are thus characterized.

Structure.—The structure of the nonelastic, or nonswelling, gels is that of a finely porous "sponge," comparable to pumice

stone or the clay of a flower pot but with pores infinitely finer. The silica gel is a mass of sand shot through with myriads of ultramicroscopic capillaries (Fig. 90). The tremendous amount of surface that these pores display is the physical basis of the excellent adsorbing qualities of silica gel. This gel is an ideal example of matter in the colloidal state.

The structure of elastic gels or jellies presents a much more difficult problem. Exceedingly little is known about their internal configuration, with the possible exception of cellulose.

It does not seem likely that the structure of jellies is a porous one in the same sense as is that of coagula. Hatschek states that these two kinds of gels are to be distinguished not primarily by such qualities as elasticity but by the fact that coagula, of the type of the silica gel, are porous, while the jellies (gelatin) are not.

Some conception of the conditions that any theory on gel structure must satisfy is to be had from the remarkable fact that certain solutions set to a rigid gel even though they possess but 0.2 per cent of solid matter. The most extreme case of this is the germanate gel prepared by J. H. Müller. Germanic acid and calcium hydroxide are mixed, and a firm gel results. The gel appears dry, and the beaker containing it may be inverted without loss, yet the gel contains but 0.1 per cent of solid matter and 99.9 per cent of water. That a solution containing but 0.1 per cent of solid matter will form and maintain a rigid gel is intelligible on the assumption that the structural units are long fibrous ones, linear, crystalline rods of molecular or colloidal (micellar) size. The classical micellar theory of gel structure, postulated by Nägelei, is discussed elsewhere (see pages 118 and 252).

Ultrafiltration.—A turbid colloidal solution will pass through filter paper. In order to filter out colloidal particles, it is necessary to have filters with much finer pores than those of filter paper, the best of which have pores of $1\text{ }\mu$, with 2 to $5\text{ }\mu$ for the average. Even porcelain Berkefeld and Chamberlain filters, used to rid drinking water of bacteria, have pores of 0.2 to $0.6\text{ }\mu$ and therefore above colloidal dimensions. Special colloidal filters, generally known as *ultrafilters*, have consequently been devised. Those made by Bechhold are of filter paper soaked in gelatin hardened with formaldehyde. Collodion sacs, the permeability of which can be varied over a considerable range by the addition of oil, gelatin, and albumin, make excellent ultra-

filters. In both of these cases, the ultramicroscopic pores of the fine colloidal structure of a gel serve as the ultrafilter.

Most living membranes are ultrafilters, the Bowman capsule of the kidney being an example. It serves in permitting certain substances of low molecular weight (uric acid) to pass from the blood into the convoluted tubules of the kidney yet holds back the larger colloidal particles of the blood proteins. It is difficult to escape the opinion that colloidal jellies used as ultrafilters owe this property to a fine porous, or sievelike, structure. This structure may be due not to granules, as in the case of silica, but rather to the intermeshing of long and fibrous crystalline units. That this is true is indicated by that extraordinary behavior of gels known as thixotropy.

Thixotropy.—*Thixotropy* is a most interesting and little understood property of gels, first thoroughly studied by Schalek and Szegvary in the laboratory of Freundlich in Berlin. An iron oxide sol is prepared after the manner of Graham (by adding a solution of iron chloride to one of ammonium carbonate until the flocculation is no longer redissolved; the mixture is then dialyzed and a 6 per cent Fe_2O_3 sol, ferrum oxydatum dialysatum, results). To the ferric oxide sol a few milligrams of sodium chloride are added, which causes the sol to set to a gel in a short time. This gel may be broken down simply by mechanical agitation, by shaking or stirring, and it then has the remarkable capacity to regelatinize into a gel as firm as the original one; the process may apparently be repeated any number of times. The gel is so firm that the beaker containing it may be turned upside down and the gel remain within. The sol—the liquid form—is so fluid that it flows like a thin oil. The phenomenon has been named thixotropy. Börjeson, working in Svedberg's laboratory in Sweden, observed the similar behavior of an alcoholic cadmium sol of only 0.2 per cent concentration. The simplest experiment in thixotropy is that done with bentonite or colloidal clay. If a few milligrams (the amount need not be exact) of sodium chloride is added to a test tube full of 10 per cent bentonite and vigorously shaken, a colloidal dispersion results which will set to a gel in a few minutes and can then be broken down by shaking, after which it will again set. The process may be repeated any number of times. The mechanism by means of which a thixotropic gel may repeatedly reform after breaking down is not

known, but it is possible that so-called van der Waals forces—forces that act at greater distances than usual intermolecular ones and probably account for a number of phenomena involving relatively loose bonds such as adsorption—may serve as attractive forces sufficient to pull together, as it were, the isolated structural units of a collapsed gel.

Thixotropic phenomena occur in protoplasm. The mere stirring of highly viscous protoplasm will often reduce its consistency. The elaborate mitotic figure of a dividing egg after fertilization, with astral rays and spindle, maintained by structural properties of the protoplasm may, when subjected to pressure, collapse with great suddenness and reduce the egg to an undifferentiated mass with no vestige of the former mitotic parts; mechanical disturbance has caused instantaneous and complete collapse of the internal arrangement of the cell material.

The Liesegang Phenomenon.—The properties of gels are such as to give them some of the characteristics of solids and some of liquids. A gel may possess the rigidity of a solid and yet permit soluble substances to diffuse through it as quickly as in liquids. The rigid quality of a gel permits precipitates, when formed within it, to be held in place. Substances that form precipitates while diffusing through a gel leave in the gel striking formations which closely parallel certain processes in nature, both in appearance and very probably in manner of formation. The precipitates may be crystals irregularly distributed, which, when at their best, present one of the most beautiful of colloidal phenomena (*A* and *B*, Fig. 91). Hatschek has made very attractive crystals of gold scattered in silica gel. Gold chloride is first added to the gel. A reducing substance (sodium sulphide) is allowed to diffuse in from the outside; the chloride is reduced, and gold crystals result. In many instances, the crystal formation occurs rhythmically so as to produce bands (*A*, Fig. 91), which, when glucose has been previously added, may number more than a hundred within 8 cm. When precipitation in gels is rhythmic, so as to produce a series of concentric rings or bands, it is known as the *Liesegang phenomenon* (*C* and *E*, Fig. 91; *A*, Fig. 92).

Rhythmic precipitation was discovered by Liesegang in 1896. His original experiment consisted in coating a glass plate with a film of gelatin which contained a small amount of potassium

dichromate; a drop of strong silver nitrate was put in the center. As the silver nitrate diffused into the gel and met the dichromate,

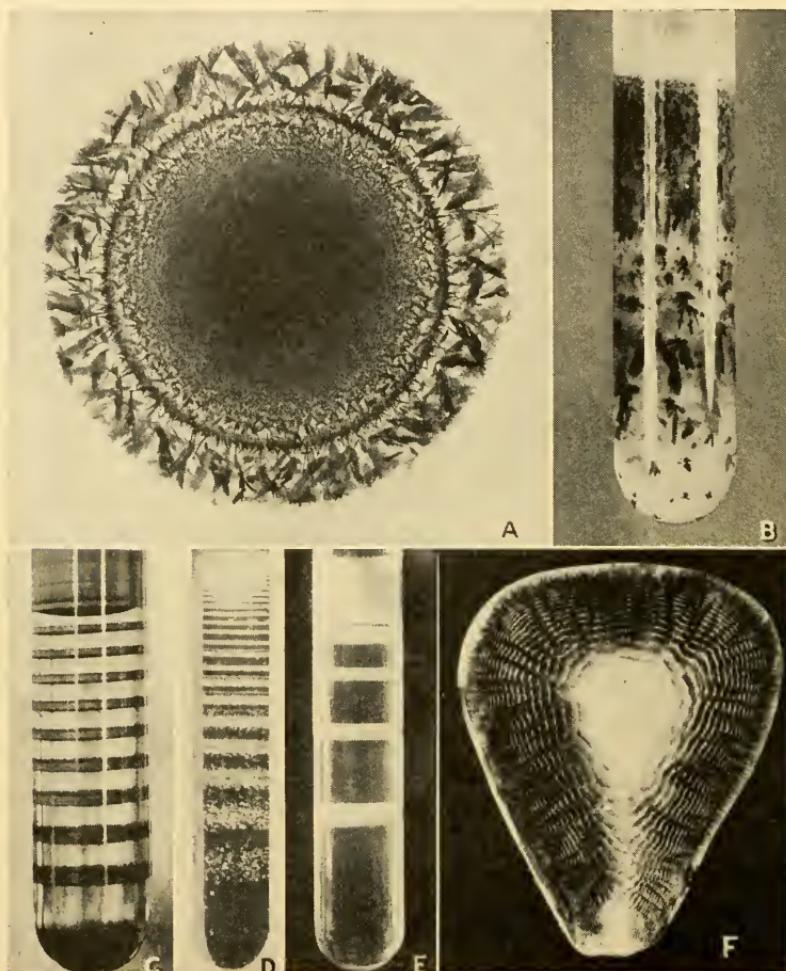


FIG. 91.—*A*, Crystals of basic mercuric chloride in a silica gel produced by allowing mercuric chloride to diffuse into a gel of basic reaction, on a plate; *B*, the same in a tube; *C*, rhythmically produced disks of silver dichromate ($K_2Cr_2O_7 + AgNO_3$, with a little potassium citrate in the gel); *D*, like *C*, but showing scattered granules as the disks become irregular; *E*, ammonium chloride disks produced by ammonium hydroxide diffusing into a gelatin gel containing magnesium chloride; *F*, rhythmic banding of lead iodide in a gel in imitation of agate.

silver chromate was precipitated. There is no evident reason why the precipitated silver chromate should not be evenly distributed and give to the gelatin plate a fine granular appear-

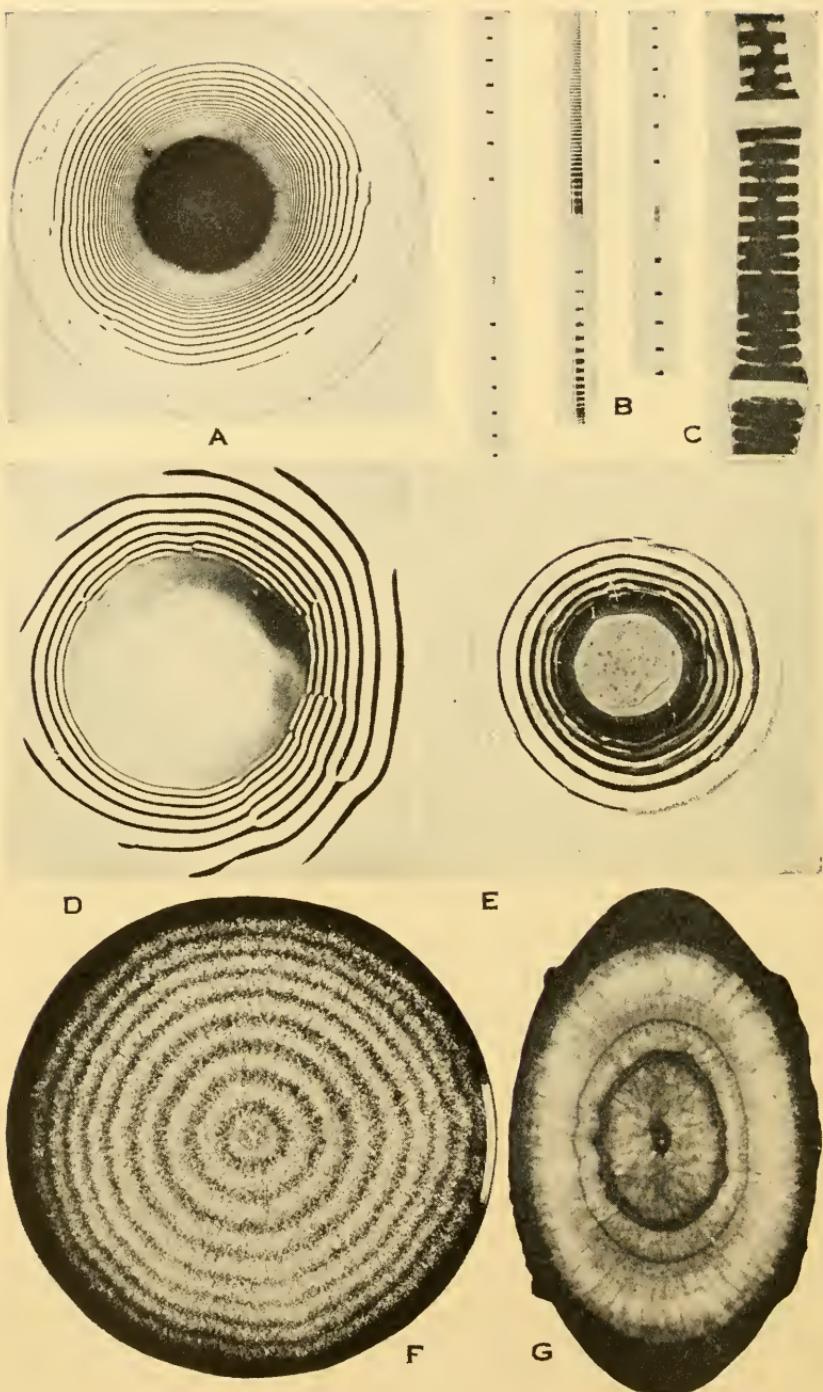
ance, but instead, the chromate is deposited in concentric rings at ever increasing distances (*A* and *D*, Fig. 92). To explain this peculiar phenomenon several theories have been advanced, one of which invokes supersaturation. The silver chromate is presumably carried along in the process of diffusion until a point of supersaturation is reached, when the whole of the precipitate is deposited and a ring is formed. The increase in distance between rings is due to depletion of the silver as it diffuses outward and unites with the dichromate. As the process proceeds, the precipitation may become less and less periodic, until at the end it is scattered uniformly through the gel. In *D*, Fig. 91, small granules of the precipitate are to be seen scattered near the bottom of the tube where the bands are becoming less and less clear-cut. Perhaps no one theory will explain all forms of precipitation in gels. Subsequent solubility of the precipitate may be a factor.

One occasionally chances upon variations in Liesegang phenomena which are very difficult of interpretation. Double banding is one of these. A strong solution of potassium citrate in a 1 per cent agar gel containing potassium dichromate will, upon the addition of silver nitrate to the surface of the gel, produce a band of white silver citrate, followed by a neutral zone; then a reddish brown band of potassium dichromate, followed again by a neutral zone, a band of silver citrate, and so on. But most remarkable is the rare occurrence of a continuous spiral (*E*, Fig. 91), even though no change in technique has unwittingly taken place. For this extraordinary behavior no explanation is known. Spiral structures often occur in nature (Fig. 14).

Another neat example of rhythmic precipitation is the rings formed in fine capillaries (*B*, Fig. 92); indeed, where the capillaries are very small, no gel is necessary to hold the rhythmically produced precipitate in place.

Liesegang pointed out the possible relationship of rhythmic banding in gels to similar phenomena in the mineralogical and geological worlds. Laboratory productions of banding may be precise reproductions of natural agates (*F*, Fig. 91).

Lloyd has observed the formation of Liesegang rings in the vacuoles of living plant cells when stained (with neutral red and other reagents). Either the contents of the vacuoles are a gel,



For caption see following page.

or, more likely, the rhythmic precipitation is an example of the fact that banding may occur in liquids when it takes place in capillaries; this qualification is fulfilled by the living cell. The capillary forces present there are sufficient to hold the rhythmically produced precipitates in place. This is nicely illustrated by Lloyd in artificially produced banding in the hairs (trichomes) of plants (*C*, Fig. 92).

The applicability of the Liesegang phenomenon to biological processes is speculative, but there is no apparent reason why it is not the cause of banding so common in animals, such as the markings on butterfly wings and the banded colors of fish and the feathers of birds (pheasants). When Hatschek spoke of Liesegang rings before the seminar at Bateson's laboratory near London, Bateson asked if biologists could assume that the stripes of a zebra were a Liesegang phenomenon. The remark was taken humorously at the time, but Lloyd has since considered it seriously. He says that whatever the cause of the parallel lining on the zebra may be, it looks most suspiciously like rhythmic precipitation. The similarity is especially marked when certain irregularities occur in gel banding (*D*, Fig. 92).

The banded colors of agates, fish, and birds, and the concentric rings in certain fossil remains indicate that the Liesegang phenomenon is rather common in nature. The anatomist Sweet has made an interesting collection of gallstones, which, on cutting and polishing, show in some cases remarkable rhythmic markings (*G*, Fig. 92). Their production is probably a typical Liesegang phenomenon due to the gradual infiltration of bilirubin (the surrounding pigment) into the calcium-cholesterol (a gel-forming monoatomic alcohol) matrix of the gallstone. The precipitate formed, in concentric rings, is calcium bilirubinate. The cholesterol is then presumably shifted from a colloidal to a crystalloidal state, the final structure of the gallstone being crystalline.

FIG. 92.—*A*, Liesegang rings of silver chromate precipitated by silver nitrate diffusing from the center into the gelatin gel containing potassium dichromate (*Liesegang's original experiment*); *B*, silver chromate bands formed in capillaries; *C*, Liesegang rings artificially produced in the living hair (trichome) of a plant (*from F. E. Lloyd*); *D*, rings produced as in *A*, illustrating irregularities; *E*, a silver chromate spiral precipitate in an agar gel; *F*, fungus (*Cephalothecium*) growing in culture and forming concentric rings of fruiting bodies which have nothing in common with Liesegang rings except rhythm (*from G. G. Hedgcock*); *G*, gallstone (twice natural size) showing Liesegang rings of calcium bilirubinate produced by bilirubin diffusing into the calcium-cholesterol base of the gallstone (*from J. E. Sweet*).

There occur in nature a number of examples of concentric bands which look suspiciously like Liesegang precipitates. This is true of the annual rings of trees; the rings formed by fungi, especially when grown in culture (*F*, Fig. 92); and the extraordinary banding caused by fungi on the trunks (bark) of trees. These processes are not to be confused with the Liesegang phenomenon; they are quite distinct from it, except that both are examples of the widespread tendency of natural processes to function rhythmically.

The Gel Qualities of Protoplasm.—Many of the properties and processes of protoplasm are typical of gels. Cells take up water in part by imbibition. Wood swells by imbibition. These are properties of gels. The control of the entrance of salts into the cell is determined, in part, by the outer layer of protoplasm which is a jelly. The elastic properties of protoplasm, its coagulation, and its adhesive qualities are properties of gels. (W. H. Lewis says that if it were not for the adhesive quality of cell surfaces we should all fall to pieces!) Adsorption, which plays so important a part in life, often acts in the minute spaces of a gel. Changes in the consistency of protoplasm, whether slow ones in viscosity or sudden ones in thixotropy, take place in going to and from the gel state.

That structure of protoplasm upon which its organization as a living system primarily depends is essentially the structure of a jelly. Protoplasm possesses some of the properties of liquids—of a sol—thus, it flows and rounds up under the influence of surface tension, but its gel qualities are more marked. Though often fluid, and though superficially an emulsion, protoplasm is primarily and fundamentally a lyophilic colloidal system, that is to say, a jelly.

CHAPTER IX

SURFACE TENSION

Few physical forces have been resorted to more frequently in explanation of vital processes than has surface tension. While speculation has gone too far in most instances, there are undoubtedly many protoplasmic phenomena which involve surface-tension changes, some few of which may in part be determined by such changes.

Surface tension is an expression of *surface energy* and is present at all *interfaces*. The “surface” of water is an interface between water and air; that it is under tension may be demonstrated by pouring more water into a glass already full to the brim until the surface is curved above the edge of the glass. The water that stands above the brim is held there by a *surface-tension membrane*. If a needle is carefully lowered on to the surface of water, it will remain in suspension (if slightly oily). The surface film of the water holds it there. Once below the surface, it rapidly falls. When droplets of water are being formed, as when dripping from a faucet, they grow larger and larger until they suddenly fall. They break loose at the moment when the surface film can no longer hold the water within them. When free in the air, liquid droplets assume a spherical shape. Their suspension at the mouth of the faucet, their size before falling, and their spherical shape when free are all determined by the tension of the water at their surface. What is true of the surface of liquids when in contact with air is also true of liquids in contact with other liquids with which they do not mix and of liquids in contact with solids. In all cases, a surface or interfacial film is formed.

Unbalanced intermolecular attraction at the surface is the physical basis of surface tension. It results in a crowding of the molecules there and the formation of a film (Fig. 93). A molecule within a liquid is attracted by, and itself attracts, all the molecules surrounding it at not too great a distance. Such a molecule would, if uniform distribution existed, have in its immediate neighborhood 12 or 14 others, 6 of which would be visible

in an optical section through the center of the liquid (*M*, Fig. 93). But a molecule at the surface has none of its own kind above it and is therefore subjected to an unequal attraction (*N*, Fig. 93). As a result, all surface molecules are pulled one way or the other; if on to adjoining foreign matter, adsorption results; if into their own mass, as usual at liquid-air interfaces, there results a closer packing of the molecules at the surface than exists deeper within the liquid. This condition at the surface brings about the formation of an elastic film which is capable of supporting a steel needle, of pulling a free droplet into a sphere, and of holding up a column of water in a capillary tube.

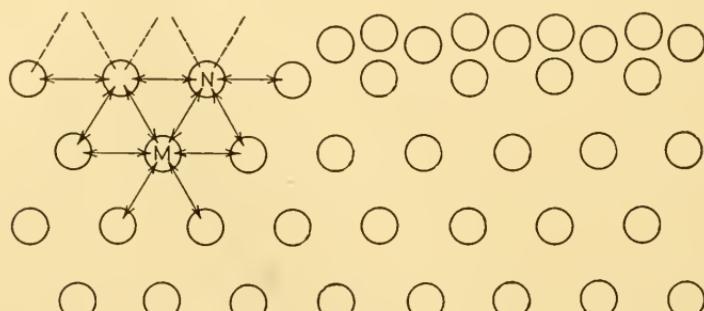


FIG. 93.—Diagram of the distribution of molecules within and at the surface of a liquid.

When water and oil are in contact, there may result two interfacial membranes (one of water and one of oil), a single composite membrane, or a transitional zone from pure water on one side to pure oil on the other. Which condition prevails cannot be said.

That a spherical or circular shape is "striven" for by surface membranes is due to the law of minimum surface which is a corollary of the law of minimum energy. The second law of thermodynamics states that all systems "strive" toward the state of least free energy—they tend to run down. A droplet assumes the shape of a sphere when free, because the sphere, among all possible shapes, encloses maximum volume with minimum surface. (There are some slight exceptions to the rule that the circle and sphere enclose the maximum area or volume with minimum circumference or surface. The surface-tension film of a liquid in a tube has, in section, the shape of a catenary. This is not the segment of a circle, but it is a minimum surface.)

There are a number of ways of evaluating surface tension. The tension in the surface of a liquid can be compared with that in the surface of water, taken as unity. The size of drops falling from a tube depends upon the tension of their surface film; consequently, the size of a drop of liquid of unknown surface-tension value will bear the same relationship to the size of a drop of water (coming from the same opening) as does the tension of the one to the other. This is true provided the liquids weigh the same; if they do not, then their specific gravity must be taken into account. It is more convenient to count drops than to measure their size; the result is the same, if the total amounts of the known and the unknown liquids are identical.

The instrument for measuring surface tension by drop counts is known as a *stalagmometer* (a "drop meter"). It consists simply of a capillary with a flattened mouth and a small reservoir (Fig. 94) and is the invention of the German I. Traube. To him do we owe the initiation of the impetus to surface-tension studies which has gone so far in biological reasoning.

If 1 cc. of water yields 30 drops, then each drop has a volume of 0.033 cc. If 1 cc. of alcohol yields 105 drops, then the volume of each drop is two-sevenths of that of water; therefore, the surface tension of alcohol would be two-sevenths of that of water, provided the two liquids weighed the same; but alcohol is lighter than water, and the weight, not the volume, of a drop is a measure of the tension of its surface film. The following formula evaluates the surface tension of a liquid on the basis of the factors involved when a drop falls from a tube:

$$T = \frac{n \cdot D \cdot t}{N}$$

where T is the surface tension of the unknown liquid; n , the number of drops of water; D , the density of the unknown liquid; and t , the surface tension of water. If the tension value of water is taken as unity, then, on the basis of the above formula, with 30 drops of water and 105 drops of alcohol and a density of 0.79 for alcohol, the surface tension of alcohol becomes 0.226.

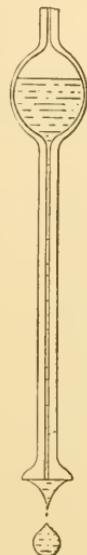


FIG. 94.—
A stalagmometer.

Values of surface tension relative to water are satisfactory, but it is better practice to have absolute values expressed in terms of the unit of force the dyne, which is the force that will produce in one second a change in velocity of a centimeter per second in one gram. On this basis, we can define surface tension as the tension exhibited by the free surface of liquids measured in dynes per centimeter. The formula $F = lT$ expresses surface tension in terms of the total force F along a line of length l on the surface of a liquid the surface tension of which is T .

Some surface-tension values in dynes per centimeter are:

Water = 75.6 at 0°C.	Ethyl alcohol = 21.7 at 20°C.
Water = 72.8 at 20°C.	Olive oil = 33.5 at 20°C.
Water = 68.6 at 50°C.	

The absolute surface tension of a liquid can be measured by the Maxwell frame. A film of, say, soap formed across a frame has two surfaces so that a pull exerted upon it by a wire with attached weight is sustained by two surface membranes. If the wire is 0.5 cm. long, then it pulls against 1 cm. of surface and measures the membrane tension per unit of length. This *force* is expressed in *dynes*. The *work* done is *force times distance* and is expressed in *ergs*. An erg is the work accomplished by one dyne in one centimeter. The force (72.8 dynes per centimeter for water at 20°C.) is the surface *tension*; the work (72.8 ergs per square centimeter for water at 20°C.) is the free surface *energy*.

Surface tension may be measured in still another way—by comparing the height to which a liquid rises in a capillary tube with that reached by water. Water wets glass; therefore it will climb up the surface of the glass and in so doing form a curved *meniscus* (Fig. 95). If the glass is a fine tube, the water will climb within it, until the weight of the column of water just balances the surface tension of the meniscus. The absolute surface tension is expressed in dynes with the aid of the formula

$$T = \frac{r \cdot h \cdot D \cdot g}{2}$$

where T is the surface tension; r , the radius of the tube; h , the height to which the liquid rises; D , its density; and g , the value of gravity. The height to which alcohol will climb in a glass

capillary is slightly more than two-sevenths of that to which water will climb.

The most precise way to measure surface tension is with the *tensiometer*. If a ring of known circumference is attached to the end of a balanced rod on the opposite end of which is a scale pan, and if the ring is in contact with the surface of a liquid, the surface tension of the liquid can be measured by adding weights to the pan until the contact between the surface film of the liquid and the ring is broken. Searle converted such a simple tensiometer into a *torsion balance*, wherein the twist or torsion of a steel wire is the force that pulls the contact ring. Lecomte duNoüy carried the improvement further. With his tensiometer (Fig. 96), direct readings in dynes per centimeter can be

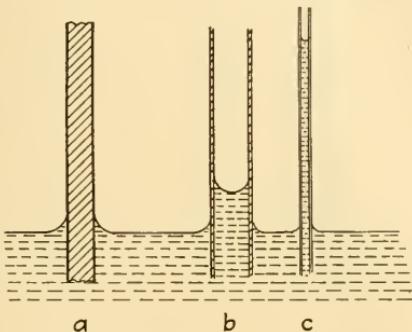


FIG. 95.—(a) Meniscus formed against glass by water; (b) the rise of water in a large, (c) in a small, capillary.

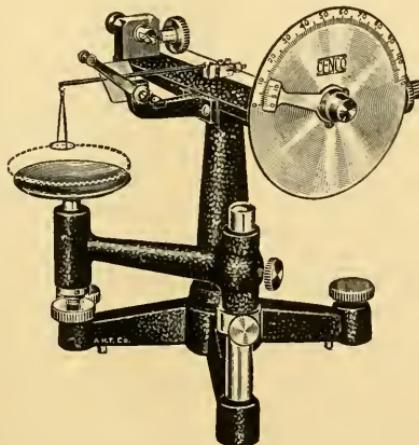


FIG. 96.—The duNoüy tensiometer. (Arthur H. Thomas Company.)

made. He has also developed an *interfacial tensiometer* for reading the tension of membranes between liquids.

Soaps and fatty substances lower the surface tension of water when added to it. The surface tension of a (saturated) aqueous solution of sodium oleate is 25 dynes per centimeter. Salts

raise the surface tension of water; the surface tension of a calcium chloride solution (of density 1.35) is 95 dynes per centimeter. Such behavior means that the surface composition of a solution differs from that of the solvent and in such a way that soaps and fats, which lower surface tension, are of higher concentration in the surface than in the interior, while of salts, which raise tension, the converse is true. This is the law of Willard Gibbs, the American physical chemist whose mathematical and physical deductions anticipated so much, though at the time (1878) they passed unnoticed. Thus does the surface of a solution or heterogeneous mixture differ both physically and chemically from the interior—physically by having a more compact arrangement of the surface molecules and chemically by having a different relative proportion of the ingredients at the surface (with the possible absence of some).

Studies in surface tension have found application in theoretical physics and chemistry. Langmuir and later duNoüy determined the size of organic molecules from their tension at surfaces. DuNoüy observed the gradual reduction in tension of the surface film of a soap solution with increase in concentration and concluded that the lowest tension value obtained of the soap solution indicated a monomolecular layer of soap at the surface. With the area of the surface and the specific gravity of the substance known, it was possible to compute the thickness of the layer and therefore the length of the soap (sodium oleate) molecule, which was found to be 12.3×10^{-8} cm. From this value and the molecular weight, the width and depth of the sodium oleate molecule were found to be 6.8×10^{-8} cm. and 7.56×10^{-8} cm. Thus may such fundamental physical problems as molecular size be attacked by quite different methods from those ordinarily employed and with corroborative results.

Surface Tension in Living Matter.—Surface tension has been regarded as the cause of many vital phenomena. Cell division, amoeboid movement, protoplasmic streaming, gastrulation, permeability changes, nerve impulses, muscular contraction, excretion, and even memory itself have been interpreted as involving, if not primarily due to, changes in surface tension. That some of these speculations are, in part, true we must acknowledge, because there is no physical force more universally present and operative in liquid systems than surface tension,

but biologists now feel that there has been undue enthusiasm for surface tension as the causative agent of certain vital processes. That surface tension is a very significant force in both the living and nonliving world is evident from the fact that if 1 cc. of water is sprayed into spherical droplets 0.01μ (0.00001 mm.) in diameter, the total area of the droplets will be 6,000,000 sq. cm. The free surface energy of this area, at room temperature ($20^{\circ}\text{C}.$), would be 218,000,000 ergs, or 10.5 cal. The extraordinary activity of colloidal systems is due to this tremendous increase in surface over the solid state.

S. Mudd has developed a neat technique, involving an interfacial film, for the study of the passage of bacteria and blood cells through surface-tension membranes. Water and oil are brought into contact on a microscope slide; the cells to be studied are suspended in the water. The red blood cell passes from the water into the oil through the interfacial film. The tension of the latter stretches the blood cell into the shape of a lens. Normal bacteria, belonging to the so-called acid-fast or lipoid-coated group, pass readily into the oil and hence are hydrophobic because of a fatty coat. After the fatty layer from the bacteria is removed, they no longer pass from the water into the oil, for their surface is now presumably protein in nature. Bacteria or red blood cells when coated (sensitized) with so-called antibodies from immune serums do not pass into the oil; their coating (antibody) is now globulin.

An interesting and somewhat amusing application of surface tension in the living world is the use to which water insects put the surface film of water. Ramsden cites the case of various small aquatic animals which rest upon or cling to the surface even though they are heavier than water. The black, hairy insect *Podura* frisks about on the surface of a pond; the larvae of the gnat hang head downward suspended from the water surface by means of their breathing tubes. Entomostraca, which normally live within water, are unable to get back into it when by accident they are caught in the surface; they float helplessly on their side until they starve to death or are blown ashore.

When an amoeba puts out a pseudopod (Fig. 24), there possibly takes place a reduction in the surface tension of the protoplasm at that point, due to some internal reaction or (less likely) an external change. The internal (imbibition or turgor)

pressure of the cell would then force a protrusion out at the point where a weakening in the tension of the membrane has occurred, just as a weak spot in a toy balloon gives rise to a protrusion there. In such a way may a pseudopod be formed. Advance would take place as follows: A pseudopod protrudes owing to reduction in surface tension and clings to the substratum; increase in tension of the membrane now draws up the rear portion of the amoeba; the process is then repeated. Berthold (1886) was the first to advance such a theory, but Jennings later came to the

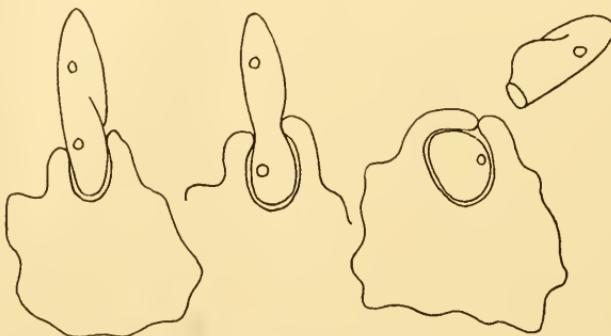


FIG. 97.—The severance of a Paramoecium by Amoeba. (*From S. O. Mast and F. M. Root.*)

conclusion that the locomotion of Amoeba is demonstrably not due to a local decrease in surface tension on the side toward which the animal is moving.

Another process ascribed to surface-tension forces is that of the ingestion or engulfing of food by Amoeba. When by chance Amoeba meets its prey, it surrounds it with two pseudopods. The victim thus caught may be taken in whole and pocketed in a spontaneously formed food vacuole, or just a bite may be taken out of it (Fig. 97). The unicellular animal Paramoecium, on which Amoeba feeds, maintains a permanent shape. It must, therefore, possess a more rigid pellicle than does Amoeba, which is always changing form. How then, is it physically possible for Amoeba with its jelly arms to pinch a firm paramoecium in two? Surface tension was presumed to be the force involved, but Mast and Root made some calculations. They found that to cut a paramoecium in half with a fine glass fiber requires a pressure of approximately 9 mg. A reduction in

surface tension of at least 1,000 dynes per centimeter at the tip of the pseudopods would be necessary if the pseudopods of an amoeba are to have the same cutting capacity as the glass fiber. The surface tension of protoplasm, according to estimations by Czapek, is two-thirds that of water, or approximately 50 dynes per centimeter. Surface tension is, therefore, according to Mast and Root, probably at best an insignificant factor in the process of feeding by Amoeba. This is probably quite true, yet surface-tension changes may be involved in other ways than by merely pinching in two. The cutting may be preceded by digestion of the surface of the paramoecium.

Far more complicated vital phenomena than the movement and feeding of Amoeba have been attributed to surface tension. But before considering these, about which we must profess almost complete ignorance, let us see if there really are one or two processes associated with life which are indubitably the direct expression of surface forces. The simplest conceivable event which may be due to surface tension is the rounding up of a droplet of protoplasm. The egg of a starfish or seaweed is round probably because of surface forces. D'Arcy Thompson says that when a "bud" appears on yeast (Fig. 98), it does so because at a certain part of the cell surface the tension has diminished; the area at that portion expands, and the bud will be rounded off into a more or less spherical form. But he points out that the parent yeast cell is not round and that it is only the incipient cell wall which behaves like a fluid drop. The important point is not how an asymmetrical shape is maintained but how it is acquired. An ellipsoidal form, like that of an old yeast cell, is maintained because the wall is a rigid gel. The lack of symmetry was acquired while the wall was still fluid or plastic, when surface-tension forces could therefore still operate. Structural features in protoplasm, such as will account for elastic qualities (page 247), may be responsible for most protoplasmic phenomena which have been attributed to surface tension. The laws of surface tension are based on the behavior of pure liquids and true solutions. Protoplasm is certainly not the former, and there is little reason to believe that it is the latter. When a droplet of water or oil rounds up, surface tension is responsible, but a droplet of proto-

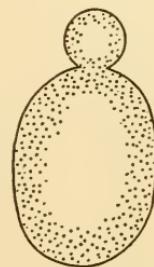


FIG. 98.—A budding yeast cell.

plasm is subjected not only to surface tension but to a uniformly distributed *tensile*, or *elastic*, quality of the surface layer. Not only will this property accomplish the same result as surface tension in rounding up the incipient (fluid) cell wall, but it gives a better mechanical basis for interpreting asymmetry, for it owes its existence not to surface forces but to structural features. If the droplet of protoplasm happens to be an amoeba, it does not always round up into a ball any more than does the less active yeast cell. Here surface forces are opposed. The Amoeba assumes any shape that it "wants" to. It increases its surface greatly.

Fission, or the division of minute unicellular organisms such as Protozoa, blue-green algae, and bacteria, may be the result of surface activity in part. One can imagine a cell with a membrane of relatively uniform tension throughout. Owing to chemical changes within, the surface tension of the membrane increases along a narrow band encircling the cell like a belt, until the cell is pinched in two, just as would be the case if one put a heavy rubber band around the imaginary equator of a toy balloon. This may happen in simple cell division or fission, but there is no conclusive proof of it. And thus it is with all our surface-tension theories of vital processes.

A Dublin physicist, Fitzgerald, was apparently the first to suggest that muscle contraction is a surface-tension phenomenon. Since then, others have advanced the same hypothesis, without, however, being in agreement on whether the contraction involves a decrease or an increase in surface tension. Fitzgerald argued that if a mammalian muscle fiber is 0.000125 cm. ($= 1.25 \mu$) in thickness, there would be about 500 m. of circumference of fibrils per square centimeter of cross section of muscle. This, with a surface tension equal to that of water, would give a disposable force of 4 kg. as against the estimated value of 7 kg. for muscle. By allowing for a surface tension less than that of water (Czapek's estimation for protoplasm was a tension value two-thirds that of water) and a smaller diameter of the fibrils, a much larger force is made available.

Bernstein argued along the same line and went to the trouble to measure the size and estimate the number of muscle fibers, to see if there was energy enough in their surface to account for muscle contraction. He took the semimembranosus of a frog,

determined its absolute force, embedded it in celloidin, and found the average radius of each fibril to be 9.994×10^{-5} cm. From this he calculated that in a square centimeter there would be 31,460,000 fibrils, which, on the basis of data for elasticity and absolute force, gives 0.022 gram as the value of the surface tension of the fibril at its fibril-sarcoplasm interface—a value near that of oil at an oil-water interface. Such hypotheses are tested out by experimentally measuring the force generated by muscle contraction. This should equal the energy liberated as a result of change in surface tension. The energy is the product of the surface tension and the diminution of the surface due to contraction. If the surface energy liberated is found to be too small, a more complex structure of the muscle fiber is postulated in order to get more surface. Such an assumption is justified, but there is no conclusive evidence to support the theory as a whole. Other postulates are just as convincing. Electrocapillary forces are at play. Differences in electrical potential cause deformation (*e.g.*, as in the case of a mercury drop) and, by thus altering form, produce shortening, or contraction. Osmotic pressure (*i.e.*, turgor) and imbibition pressure are forces available to actuate muscles. Plant "muscles" (motor cells) are "pumped up" with water, establishing turgor pressure, which is later relieved when the "muscle" collapses (and the plant wilts). Animal muscle may have the structure of a sponge or jelly, a porous meshwork, into and out of which fluid passes. While the various theories on the part that surface tension plays in muscular contraction are largely speculative, it is possible that surface tension is yet a factor in muscle action.

Macallum, in an exposition on surface tension and vital phenomena, closes with a speculation on the physical nature of psychic functions. He says that sensation, that is to say, the nerve impulse, is fundamentally and primarily a result of alteration in the surface tension of nerve cells and their processes. He believes that he can thus explain anesthesia and narcotism. Chloroform, ether, and alcohol lower the surface tension of cells, especially nerve cells, and so make them incapable of receiving or transmitting a nerve impulse. Memory itself, Macallum concludes, may arise from the adjustment of the surface tension of the cells in centers of the cerebral cortex. (Actually, we know nothing about the mechanism of memory.)

We can only hope that some few of these speculations are, in a small measure, true. That surface forces are everywhere present in living matter, from the minute but multitudinous particles within protoplasm to the surface of the organism as a whole, there can be no doubt, but we are still almost wholly ignorant of the part that they play in vital phenomena. Physical and chemical laws are applicable to protoplasm, but the latter system involves infinitely more variables than the systems for which the laws were stated. It is the task of the biologist to ascertain how far the laws are applicable.

CHAPTER X

ADSORPTION

As we turn from one physical force to another which is operative in living systems, each appears to be more important than the other, either in reality or because more attention has been paid to it. Adsorption has certainly received its share, if not a major share, of attention. This is in large measure due to the application of colloidal principles to biological problems. While possibly, as in the case of surface tension, there has been too much confidence in theories, it is nevertheless true that adsorption is a phenomenon of wide occurrence in the living as well as in the nonliving world. Adsorption has to do with surface, and it is at surfaces that chemical reactions take place; the former is in many instances the precursor, if not the determiner, of the latter. As protoplasm offers many surfaces (the protoplasmic emulsion alone is responsible for innumerable boundaries), then adsorption must play a large role in the activities of the living cell, simply because of the chemical reactions that it initiates.

White cotton material dipped in a dye assumes the color of the dye if conditions are favorable. The union between the cotton and the dye may be a weak one, as when one washing severs it; or a firm one, if repeated washings do not sever it. In the first case, a bond of some sort must have been established; otherwise the cotton could not have taken the dye out of its solution. In the second case, though the bond was a firm one, there is no conclusive evidence that a new chemical compound was formed. The dye appears merely to be securely held at the surface of the cotton without having gone into chemical combination with it. This phenomenon is *adsorption*, or *surface condensation*. The substance at the surface of which adsorption takes place is the *adsorbent*. (Surface here means not only the visible outer surface but the infinitely greater inner surface of all the minute pores or particles which form the porous structure of the adsorbent.)

A precise definition of adsorption is difficult to give and is usually unsatisfactory. It is best to define and use the term simply to mean the concentration of one substance at the surface of another, or, as Freundlich says, *the loose fixation of particles at surfaces.*

The origin of the word "adsorption" is somewhat obscure. Probably it arose from "absorption." Adsorption is commonly distinguished from absorption by the fact that the former has to do with purely surface forces, while the latter is concerned with capillary forces; for example, a dye is adsorbed to the surface of charcoal, while water is absorbed by (in the capillaries of) a sponge or blotter. This distinction is, however, weak and arbitrary, for the ultimate forces in both are similar. Furthermore, the chemist technically uses absorption in quite another sense, *viz.*, to indicate the taking up of gases by liquids, which involves essentially a going into solution. Yet it seems permissible to distinguish between the adsorption of a gas by charcoal, through condensation of it on the surface of the charcoal where it is held by adhesion; and, on the other hand, the absorption of water by capillaries which hold the water in pores by capillary action or cohesion. The term *sorption* has been introduced to avoid confusion. It implies the taking on of a substance without indicating the mechanism (though usually denoting that no new compound has been formed). It is a question whether another term for what is ordinarily called adsorption is a help.

A few examples of adsorption will give a better understanding of the phenomenon in a general way and of the mechanism involved than do terms and definitions. A neat experiment demonstrating that adsorption is a surface phenomenon is the following: Very dilute soapy water is colored with methyl violet, thoroughly shaken, and the foam pipetted off. Shaking is repeated, and the foam again collected. After some dozen repetitions of this process, the color of the liquid which has settled from the foam collected will be considerably darker than that of the original solution. The dye has each time been concentrated on the surface of the membranes of the soap bubbles. The experiment may be done in another way, by shaking a dilute solution of albumin and drawing off the foam. After repeating the operation a number of times, that portion drawn off will be found to be richer in albumin than the watery solution

remaining. Not only do these experiments illustrate that adsorption is a surface phenomenon, but they prove the rule of Willard Gibbs that substances which lower surface tension accumulate at the surface.

The use of charcoal for removing moisture and odors from the air and color from liquids (brown sugar is made white in this way) is a well-known commercial practice. Charcoal as a gas filter, for purifying air for breathing, played an important part in the World War. It filled the gas masks of soldiers, and the more compact the wood (coconut shells and peach pits) the greater the number of pores and adsorbing surface per volume. Silica gel, which, when wholly dry, is a hard and very finely porous aggregation of sand particles (Fig. 90), is one of the most efficient of commercial adsorbents (for removing odors in refrigeration, for drying air, etc.). Air blown into a steel blast furnace must be dry. To accomplish this, it is passed through charcoal or silica gel which adsorbs the moisture suspended in the air. The adsorbent may be used again after reactivation by drying at high temperature.

Negative and Selective Adsorption.—The terms *negative* and *selective* adsorption are useful at times but are inexact, as no adsorption is negative, and all is selective.

Negative adsorption is said to take place when a solution becomes more concentrated (in regard to the solute) after being shaken with an adsorbent. Negative implies that the solute has been pushed away by the adsorbent, but even this unlikely event would not add more of it to the solution. It is quite evident that what has actually happened is (selective) adsorption of the solvent (water), leaving more solute *in proportion* to the solvent than there was at first. Negative adsorption exists solely by definition. It is similar to negative pressure (suction) and negative surface tension. A negative force exists because there is another positive force working in the opposite direction. So it is with negative adsorption. One *talks* of adsorption and *measures* concentration. With respect to that something measured which is a *ratio* and not a thing or event, the result is negative; but with respect to that something which is a process—a physical happening—the action is positive.

Reduction in the concentration of an acetone solution when shaken with charcoal is a classical example of adsorption; it is

said to be selective because charcoal adsorbs acetone more readily than it does water.

A striking demonstration of so-called selective adsorption is that of the dyeing of silk or cotton in an ammonia solution of fuchsin. The dye, fuchsin, is red, but the ammonia solution of it is colorless. If a piece of silk is put into such a colorless solution of fuchsin, the silk is stained red through (selective) adsorption by the silk of the fuchsin molecules in the solution.

Selective adsorption is better illustrated in the process known as *capillary analysis* in which the selective adsorptive powers of cellulose (filter paper) are used to separate substances; it is a routine laboratory procedure. If a piece of filter paper is placed with one edge in a solution of two dyes which are not adsorbed with equal readiness, then the more strongly adsorbable one will not ascend so high; it is taken up more rapidly. If the mixture is of methylene blue and eosin, there occurs first a blue zone containing a mixture of both dyes, and above a red zone of eosin alone. (Capillary analysis is probably not a very exact method. More than simple adsorption is involved. The calcium content of the paper is a variable that will affect the result.)

Possibly the best illustration of selective adsorption is that of ions which can as a result be arranged in a so-called Hofmeister series.

Hofmeister Series.—Preliminary reference is here made to the Hofmeister series (page 445), as it is in respect to adsorption that such series particularly appear. Rona and Michaelis found that salts of the same cation are adsorbed from water by charcoal in the following order:



which is the same order in which Hofmeister found them to affect the swelling of protein gels, and Tröndle observed their penetration into living cells.

The Chemical and Physical Constitution of the Adsorbent.—It has long been customary to say that the adsorption constant of an adsorbent; *i.e.*, its efficiency or capacity to adsorb gases or substances in solution, is not influenced by its chemical nature but only by its physical constitution; in other words, it makes no difference whether the adsorbent is charcoal, meerschaum, or silica gel, if the structure (determined by porosity or surface

exposed per unit volume) is the same in each case. This rather unexpected fact emphasizes the purely physical nature of adsorption and leads one to believe that a strictly chemical union does not take place. That the chemical nature of the adsorbent is not (usually) a factor is shown by the experiment in which the order of adsorbability of a series of substances (alcohols) is preserved unchanged in substituting one of three adsorbents, *viz.*, blood charcoal, talcum, or sulphur. The *order* of the series of substances adsorbed remains the same; therefore, the chemical constitution of the adsorbent is not a factor, but the *efficiency* of the respective adsorbents differs owing to differences in their physical structure. Charcoal is fifty times as effective as talcum, and talcum more effective than sulphur. Troublesome problems in adsorption arise from such instances as the following: 12 grams of kaolin is equivalent to 1 gram of blood charcoal in respect to the adsorption of methylene blue, while 1,000 grams of kaolin is insufficient to produce the effect of 1 gram of blood charcoal in the adsorption of heptyl alcohol.

While it is the physical rather than the chemical nature of the adsorbent which usually determines its efficiency, this is not always true. Barger has shown that the adsorption of iodine by organic solids is very definitely dependent upon the chemical nature of the adsorbent. Furthermore, in the commercial process known as selective dyeing, each dye reacts with only one type of fabric. It is thus possible to get two or more colors from the same bath. A bath containing amacid milling scarlet, amanil sky blue, and S. R. A. golden yellow will dye silk scarlet, viscose rayon blue, and celanese yellow. Selective dyeing with two colors is understandable if we interpret adsorption as a phenomenon involving electric charge, but selective dyeing with three colors forces one to grant that the chemical constitution of the adsorbent is a factor.

Although the chemical constitution of the adsorbent appears to be unimportant in certain cases, this is seldom true of the substance adsorbed, as in the case of homologous series (*e.g.*, of alcohols), each member of which is taken up (*e.g.*, by charcoal) in increasing amounts the higher they are in the series (Traube's rule, which follows).

Gibbs' Law.—Willard Gibbs developed certain physical principles which have become classic. Brief reference has been made

to them, but they will bear repetition and elucidation. The scientific contributions of Gibbs underlie much of the theories of solution, surface tension, and adsorption.

Gibbs expressed adsorption reactions mathematically. They may also be expressed as a principle. If we return for a moment to our definition of adsorption as concentration at a surface, we see that if particles in a solution gather at the surface of the solution, this is as much adsorption as when particles are taken out of solution by a porous solid. Gibbs' law may be stated as follows: A substance in solution will become more concentrated at the surface if it lowers the tension there and less concentrated at the surface if it raises the tension. Soaps and fats lower surface tension; they will, therefore, be more concentrated in the surface film than within the solution. We may restate Gibbs' principle thus: A dissolved substance is positively adsorbed if it lowers surface tension and negatively adsorbed if it raises it. This is true because free surface energy tends toward a minimum, and any substance that contributes toward a lowering of the surface energy remains in the surface. If this statement is made general by saying that all energy strives toward a minimum, we have the second law of thermodynamics upon which Gibbs' principle rests.

Traube's Rule.—Gibbs told us that substances which lower surface tension will be concentrated (adsorbed) at the surface. Traube found that the reduction in surface energy, or the amount of substance adsorbed in the surface film, increases with each higher member of a homologous series of alcohols or fatty acids. Each member of these series differs by one CH_2 group from the preceding member, thus:

Alcohols	Fatty Acids
Methyl, $\text{CH}_3\text{—OH}$	Formic, $\text{H}\text{—COOH}$
Ethyl, $\text{C}_2\text{H}_5\text{—OH}$	Acetic, $\text{CH}_3\text{—COOH}$
Propyl, $\text{C}_3\text{H}_7\text{—OH}$	Propionic, $\text{CH}_3\text{CH}_2\text{—COOH}$
Butyl, $\text{C}_4\text{H}_9\text{—OH}$	Butyric, $\text{CH}_3\text{CH}_2\text{CH}_2\text{—COOH}$

Each successively higher member of a homologous series is more effective in lowering surface tension than the preceding member. It lowers surface tension the same amount when of a concentration but one-third that of the previous member.

Freundlich's Adsorption Isotherm.—*Adsorption constants* are mathematical measures of the degree to which a substance is

taken up by an adsorbent. Thus, if meerschaum, a hard, porous gel, is put in a chamber with ammonia gas, the gel will take up a definite amount of gas per gram of adsorbent (meerschaum), depending on the adsorption constant and a number of other factors including temperature and the pressure of the gas.

To characterize a substance by its adsorption constant is tacit admission that the adsorption reaction is nonstoichiometric. Van Bemmelen and others pointed out that the adsorption of substances was not stoichiometric but followed the empirical formula

$$a = \alpha c \frac{1}{n}$$

where a is the amount adsorbed per gram of the adsorbent; c , the concentration of the solution in equilibrium; and α and n , constants (α is the adsorption constant). This formula was used by Freundlich, who applied it extensively and showed that it holds in a great number of systems; it is, therefore, known by his name.

A definite quantity of charcoal takes up a definite quantity of gas at a given temperature and pressure, but the quantity adsorbed does not vary *proportionally* to pressure; that is to say, it is not stoichiometric. The equation of Freundlich expresses this nonstoichiometric relationship. The curves obtained are known as *adsorption isotherms*, because they depict the adsorption behavior—the variation of adsorption with pressure—at constant temperature. Freundlich's contribution lies chiefly in the fact that he showed the adsorption equilibrium formula to be widely applicable, but it is purely empirical and holds only within a certain concentration range.

Of more theoretical significance, yet holding less often in practice, is the adsorption formula of Langmuir. It is based on the theory that solid crystals are of a lattice structure (Fig. 143), with surface atoms having free valences each of which is available for attaching only one molecule from the surrounding gas or solution; in other words, it assumes adsorption to be stoichiometric. The rate of adsorption depends upon the number of molecules striking the surface (calculated from the kinetic theory of gases), the fraction of molecules that adhere, and the area not covered by adsorbed molecules.

Adsorption Bonds.—We have indicated that adsorption may be a molecule for molecule reaction and thus involve primary valence, or that it may be a surface phenomenon which is non-stoichiometric and involve a less firm union. That adsorption has to do with bonds differing in degree of firmness is evident from the many kinds of surface reactions that are included under it.

When smoke clings to a glass surface on being blown across it, or when glass is wet by water, the bond is a loose one, and no question of a true chemical union arises. The inert gases argon and radon are readily adsorbed by charcoal; a union involving primary valence is hardly possible here, for these gases rarely, if ever, form compounds. Between such examples of loose adsorption bonds, on the one hand, and others involving true chemical (primary valence) bonds, there are many intermediate types of union which the chemist has difficulty in distinguishing. Thus, some dyes are held very firmly, and others loosely. Congo red is taken out from its aqueous solution by filter paper. The ease with which the dye is then set free again from the paper by the addition of a little acid or alcohol is evidence of a loose union between paper and dye. Water is held by substances in varying degrees of firmness. Some water can be driven out of wet charcoal by the application of slight heat, but all the water is removed with difficulty. The force with which charcoal holds water by adsorption is very great, reaching many thousands of atmospheric pressures. Under such pressure, water is compressed to 75 per cent of its original volume, and it is one of the least compressible substances known.

This leads us to the problem of the nature of water of crystallization which was formerly regarded as adsorbed water and is now looked upon as involving a true primary-valence bond (page 422). Bragg believes the water molecules of hydrated salt to possess, at least in a large number of cases, no special clear identity; that is to say, they are not simply attached to other molecules in the solid, but rather is the crystal with the water a new compound altogether, with a new arrangement of the atoms. It does not follow that because the water is readily separable from the salt by heat and is given off as a molecule, it is existent as such in the crystal. J. A. Wilson expresses the view that adsorbed ions in general become an integral part of the surface.

We are forced at the outset to admit that all attempts to distinguish between types of adsorption are, in the present state of knowledge, futile. We cannot distinguish between electronic adsorption and primary valence, or polar and nonpolar adsorption; but that differences exist is evident; for example, the adsorption of an electrolyte by a difficultly soluble salt such as kaolin is probably different from that of a nonelectrolyte adsorbed by what we may call a "nonsalt," for want of a better term, such as charcoal. The adsorption of an ionized salt, like the chloride of methylene blue, by kaolin, involves an exchange of ions (the

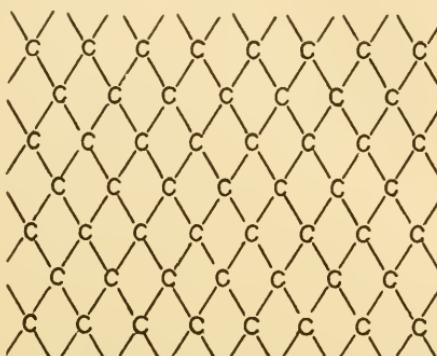


FIG. 99.—Diagrammatic representation of the free valence bonds of atoms at the surface of a block of carbon.

cation of the dye methylene is adsorbed by the kaolin; the anion, chlorine, stays in solution; in order to keep the solutions electrically balanced, the kaolin gives up calcium ions), but when charcoal takes carmine out of its solution, there is no exchange of ions.

While it is best not to distinguish too sharply as yet and to regard adsorption as any form of surface concentration, yet differences exist, and we may, therefore, consider the types of bonds that could be responsible for adsorption phenomena.

The union between atoms established by the sharing of an electron is primary valence. Adsorption may be of this type and is then said to be *electronic*. It is distinguished from primary valence in that it is strictly a surface reaction, involving only those molecules which are exposed at the surface of the adsorbent. The distinction is, however, purely arbitrary. It is illustrated in Fig. 99, which is an imaginary cross section of a block of carbon. All four of the valence bonds of each carbon atom within the

block are satisfied, but the surface atoms have exposed valences which may become satisfied through the adsorption of a foreign substance.

In the case of carbon, the unsatisfied surface forces are primary valence bonds; forces of such strength, involving, as they do, the sharing of an electron, are not frequently left unsatisfied and therefore are not usually free to take part in surface phenomena. More numerous are those looser bonds which go under the name of *coordination numbers*. These latter are significant in adsorption. Every atom in a crystal of sodium chloride is surrounded by six of the opposite kind. All atoms at the surface of the crystal lack one of their six mates; those at the edges lack two, and those at the corners lack three (Fig. 143). While sodium is united by its primary valence of one to only one chlorine atom, its coordination number of six unites it to six chlorine atoms. Were we arbitrarily to recognize but one valence bond between a sodium and a chlorine atom in the sodium chloride crystal, it would be impossible to say between which two atoms this bond exists. We are, therefore, forced to admit that each of the six bonds represented by coordination numbers is equal, and where one or more are unsatisfied, as at the surface, they are left free to unite with foreign substances. Bonds for which coordination numbers stand are responsible for adsorption phenomena rather than primary valence. The situation existing in sodium chloride is true for all crystalline substances and probably for most others; therefore, every solid has unsatisfied bonds at its surface, and upon these does adsorption depend.

The nature of the bonds expressed by coordination numbers is not fully known, but they, and therefore also adsorption bonds, may be interpreted as follows: any molecule considered at a distance is an electrically neutral body, but at close range the individual atoms, considered as ions (as they are in crystals, for example), exercise an individual electric effect which is not counterbalanced by the other atoms or ions of the molecule. For example, the water molecule is, as a whole, electrically neutral. The two negative charges of the oxygen atom are balanced by the two positive charges of the hydrogen atoms (Fig. 138). At close range, however, these negative and positive charges will exercise an influence independent of each other and thus attract and hold a body of opposite charge. This is true of all polar

molecules, or, shall we say, molecules are polar because of this electric difference. Such forces are attractive and may be responsible for adsorption phenomena. Briefly and broadly put, surfaces are electrically charged and therefore attract and hold substances of opposite charge (Figs. 80, 177).

That electric charge, in the form of stray fields of electromagnetic forces surrounding molecules, plays an important role in adsorption is evident in the behavior of *substantive* and *adjective* dyes. Acid fuchsin is negatively and basic fuchsin positively charged; the one or the other, not both, is usually adsorbed by an adsorbent; for example, kaolin is negative, and aluminum positive; the former adsorbs only positively charged (basic) dyes, and the latter only negatively charged (acid) dyes. In dyeing cotton cloth, where the fibers and the desired dye are of the same charge, a "mordant" of opposite charge is first added. It is adsorbed by the fibers and confers on them a charge of opposite sign, *i.e.*, the sign of the mordant. An acid solution gives the fibers a positive charge, and an alkaline solution gives them a negative charge. The dye of opposite charge is then readily taken on.

While forces expressed by coordination numbers present the most satisfactory interpretation of adsorption, undoubtedly other forms of attraction are involved, such as have been characterized as residual valence and forces of the nature of those of van der Waals.

Adsorption in Life.—Few phenomena characterize vital processes more than does adsorption. Freundlich states that poisoning may be due to adsorption which follows his adsorption isotherm. He had in mind the taking up of veratrine by the marine snail *Aplysia*. We are more interested in ourselves than we are in marine snails, so let us carry this identical problem over to human physiology and consider O. Warburg's adsorption theory of narcosis and anesthesia. The theory had been previously advanced by Traube and Czapek.

Following up some experiments of Freundlich's on the adsorption of oxalic acid by blood charcoal, Warburg, in searching for a possible explanation, found that oxidation of the oxalic acid into carbon dioxide and water takes place. He then proved that this inanimate oxidation process can be retarded by narcotics just as animate oxidation, *i.e.*, respiration, is retarded by them.

Furthermore, the retardation of inanimate oxidation by narcotics rises with the adsorption constants of the narcotics used (methyl, ethyl, propyl, and phenyl urethane). Apparently, the oxidation of oxalic acid into carbon dioxide and water in the presence of charcoal takes place on the surface of the charcoal, from which it may be assumed that the oxidation of sugars into carbon dioxide and water in cells (*i.e.*, respiration), takes place on the surface of infinitely small protoplasmic particles. Any diminution of these surfaces will mean a decrease in oxidation. Narcotics are readily adsorbed by charcoal and by blood particles and thus decrease the surface available for oxidation. (The blood particles that Warburg has in mind are ultramicroscopic ones.) Narcosis and anesthesia are thus regarded as a retardation of oxidation due to decrease in the free adsorptive surface of blood particles through adsorption of the narcotic. The hypothesis is a feasible one and has other facts to support it; thus, the members of the following homologous series of alcohols are adsorbed in increasing amounts as we ascend the series, and the narcotic effects also increase in this wise.

Alcohols	Concentrations Required to Cause Anesthesia of Tadpoles, M
Methyl.....	0.60
Ethyl.....	0.30
Propyl.....	0.10
<i>N</i> -butyl.....	0.04

The evidence supporting Warburg's hypothesis is offset by the fact that methanes and hydrocarbons show little or no surface activity, yet all are good anesthetics. Surface activity or adsorption rests in large measure on the polarity of the molecules involved (page 256); hydrocarbons are nonpolar and therefore not adsorbed, but they are strong narcotics. Furthermore, many substances, such as the sugars, are readily adsorbed and have no narcotic effect whatever. And, finally, the cyanides, as narcotics, are ten thousand times more effective than they ought to be if they must cover a sufficient surface. The cyanides may

function in some other manner, such as inhibiting oxidation, possibly by uniting with the catalyst iron. (For other theories of narcosis, see pages 290, 497.)

The foregoing are biological problems involving adsorption. There are many others to which references are made on the pages following.

CHAPTER XI

OSMOSIS

The shrinking of plant protoplasts away from their cellulose walls—the phenomenon known as *plasmolysis* (page 194, Fig. 106)—led botanists to study the causes underlying the pressure which keeps plant cells *turgid*. The process responsible for the *turgor* of plant cells is *osmosis*. Osmosis in the living world is most often studied in association with plant cells, but it is now recognized as a property of animal cells as well. In plants, it is the cell vacuole which is regarded as the osmotic mechanism; but in animal cells, it is the cell as a whole, thus including the cytoplasm, which is the osmotic system. How far osmosis, as a force distinct from imbibition, is operative in animal cells is still a moot question.

An Osmotic System.—*Osmosis* was discovered in 1748 by the Abbé Nollet and not again referred to until a century later, when the Frenchman Dutrochet investigated it further. He found that when a solution, *e.g.*, of salt or sugar, is separated from pure water by an animal membrane, water diffuses through the membrane more rapidly from the pure-water side than from the solution side, resulting in a rise in the level of the solution and the consequent production of a (hydrostatic) pressure on the solution side of the membrane. The Dutch and German botanists Hugo deVries and Wilhelm Pfeffer next (1877) took up the work in their studies of plasmolysis. Nowhere in science is there a more dramatic series of events than those which led from a simple botanical experiment in plasmolysis to the laws of solutions. Nägeli discovered the selective permeability of the plasma membrane; deVries formulated the principles underlying it; Pfeffer determined the pressures developed within plant cells; van't Hoff enunciated the solution laws; and Arrhenius set forth the dissociation theory out of which arose the science of electrochemistry. The last substantial work on osmosis was done in America, where Morse and Frazer made exceedingly precise measurements of osmotic pressures.

To define osmosis, and other terms associated with it, satisfactorily is difficult without reference to an osmotic system. This is due in part to an unfortunate use of terms and in part to the fact that osmosis is simply another well-known physical process, *viz.*, diffusion, taking place under certain conditions. The usual osmotic system as assembled in a laboratory consists of a solution of sugar in water separated from pure water by a parchment paper or other membrane which is permeable to the solvent water but not to the solute sugar. Water passes through the membrane in both directions but in excess from the pure-water side and therefore produces a pressure on the solution side. A membrane permeable to one kind of molecule (water) and not to another (sugar) is said to be *semipermeable*. *Selectively* or *differentially* permeable would be a more precise designation. Actually, parchment paper is permeable to sugar in solution but sufficiently poorly so, in contrast to the much more rapid diffusion of water through it, as to cause it to function as an osmotic or selective membrane. Few membranes are wholly impermeable to any molecularly dispersed substance. Even protein molecules get through collodion membranes but so slowly that the membrane acts as if it were impermeable to the solute, protein, and permeable to the solvent, water. Practically every skin from any source in nature is a selectively permeable membrane which may function in an osmotic system. The importance of membranes in vital processes rests in a large measure upon their selective permeability. The most delicate of living membranes is that at the surface of protoplasm. It determines in part the substances that enter and leave the cell. The pig's bladder is a more substantial osmotic membrane often used in the past for laboratory experimental purposes. The solvent in natural osmotic systems is water. The solute may be any water-soluble substance for which the membrane is not (readily) permeable. Salts and sugars are the most common. Organic substances of high molecular weight (*e.g.*, proteins) are not satisfactory because of the large size (colloidal nature) of their molecules.

Types of Osmotic Systems.—A common type of laboratory osmotic system is a glass thistle tube over the mouth of which a membrane of parchment paper is tightly fastened (Fig. 100). An animal membrane may also be used. Still another form is made from a sack or thimble of gelatin or collodion into which a

tube, tightly fastened, projects. A concentrated (20 per cent) solution of sugar or pure molasses is put in the thistle tube or sack, which is then immersed in water. Such an apparatus is termed an *osmometer*, or *osmoscope*. (The latter term was suggested by Ganong because, strictly, an osmometer must be capable of measuring (maximum) osmotic pressure, which the usual osmotic sack does not do.) Water enters the thistle or sack and rises in the tube, climbing higher and higher, and thus

by its own weight creates a pressure—a hydrostatic pressure. If the tube or sack is tightly corked at the top and full at the beginning of the experiment, some water will still enter and create a pressure within. There is no pressure until water has entered. This pressure causes *turgor*.

The living plant cell (Fig. 4) is just such an osmoscope, made, to be sure, of other material but functioning in the very same way and developing a pressure within the cell. The sap in the cell vacuole corresponds to the sugar solution; the protoplasmic membrane around the vacuole (or the outer membrane around the protoplast) is the semipermeable membrane. It is kept from bursting by the cell wall of cellulose. The surrounding water within the plant body, or in the soil or pond, is the outside

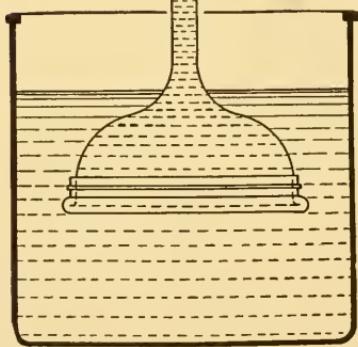


FIG. 100.—An osmotic system consisting of a thistle tube with mouth covered by a membrane; sugar solution within and water without.

aqueous medium. The pressure developed by plant cells may be very high, usually about 10 atmospheres.

A classical and picturesque demonstration of an osmotic system is that in which the selective membrane is one of copper ferrocyanide. (It was first made by M. Traube, in 1867.) Crystals of copper sulphate (CuSO_4) are dropped in a solution of

potassium ferrocyanide ($K_4Fe(CN)_6$). A precipitation membrane of copper ferrocyanide ($Cu_2Fe(CN)_6$) is immediately formed around the sulphate crystals. Water enters, but the sulphate molecules cannot get out and establish an equilibrium in concentration; consequently, the membrane is subjected to a pressure within until it bursts. This immediately exposes a new surface between the inner copper sulphate and the outer potassium ferrocyanide, which results in a patch being formed by a new precipitate of copper ferrocyanide. Again water enters in excess, turgor is produced, the membrane bursts and is repaired once



FIG. 101.—“Growing” osmotic systems with copper ferrocyanide membranes.

more. In this way, the miniature osmotic system grows (Fig. 101). It has been suggested that growth in organisms is of this sort. Some similarity there may be, but growth involves more than mere distention.

A more refined and far more secure type of osmometer is made by supporting the Traube precipitation membrane within the walls of a porous clay cup. This is done by filling the cup with copper sulphate and immersing it in potassium ferrocyanide. The precipitation takes place where the two salts meet within the wall of the cup (Fig. 102). Such a supported copper ferrocyanide membrane is not so readily broken. It may therefore withstand great pressures and for this reason has been much used for experimental work.

A copper ferrocyanide membrane has certain properties which are very similar to those of the living cell membrane; thus, it is impermeable to certain salts (copper sulphate and potassium

ferrocyanide) but permeable to others (potassium chloride). It is also impermeable to barium chloride, calcium chloride, and potassium sulphate.

The simplest interpretation of the selective permeability of membranes is that in which the membrane is regarded as a sieve with pores that let small molecules pass but not large ones.

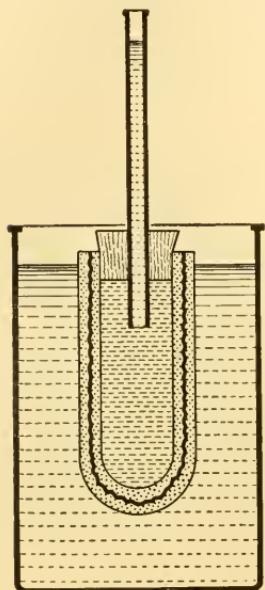


FIG. 102.—Osmotic system with a copper ferrocyanide membrane precipitated within the walls of a porous cup.

There are some difficulties with this theory, in that big molecules occasionally get through while little ones do not. Wilhelm Ostwald suggested that the electrical properties (sign of charge) of membranes may be such as to permit one type of ion (positive or negative) to pass but not the other type. Sugar, however, is not an ion. Possibly both mechanical (sieve) and electrical (charge) properties are at work (pages 281, 284).

Terminology.—The osmotic pressure of a solution is an evaluation. Actual pressure exists only when the solution is part of an osmotic system, *i.e.*, when it is confined in a sack made of a semipermeable membrane. In order to distinguish between actual pressure (*turgor*) and osmotic pressure in the sense of a rating of the solution, botanists have substituted *value* for pressure. Whether value or pressure, what we are dealing

with is a type of (osmotic) *activity*, or *tension*. Osmotic pressure is that pressure which must be applied to a solution confined by a semipermeable membrane in order to prevent the entrance of water. This pressure is the osmotic pressure of the solution, but no pressure is developed *until water has entered*, and this pressure is *not* the osmotic pressure but *turgor*.

Turgor is a distending force; it is the pressure developed within an osmotic sack and rarely equals the potential osmotic pressure of the confined solution. The movement of the water is termed *osmosis*; it is the excess diffusion of water from the pure-water side to the solution side (under one atmosphere of pressure and at the same temperature as the solution).

As water enters, the concentration of the solution is reduced. *Turgor* is increased, but *osmotic pressure* is decreased because osmotic pressure is proportional to concentration, and if concentration is changed, the osmotic pressure is changed. This makes it clear why osmotic pressures cannot be measured in terms of turgor.

As more water enters, the increased turgor opposes osmosis (excess diffusion of the incoming water). The ratio between incoming and outgoing water molecules is decreased. Ultimately, a state of equilibrium results, and osmosis ceases.

Greater concentration of sugar outside the sack will produce excess diffusion in the opposite direction from that thus far considered, and any previously existing pressure within the sack will be relieved. Diffusion in this direction, from within to without, is known as *exosmosis*, in distinction from the more usual *endosmosis*.

If the solutions on the two sides of the membrane are of the same substance (salt, sugar, etc.) and of like concentrations, no osmosis will take place, and the solutions are said to be *isosmotic* or *isotonic*. If they are of different concentrations, then the one of higher concentration is *hypertonic*, and the one of lower concentration *hypotonic*, to the other. Osmosis occurs from a hypotonic solution to a hypertonic solution and is at zero between isotonic solutions. (The story holds strictly where the two solutions are of the same kind, *e.g.*, both sugar; but where of different kinds, *e.g.*, salt on one side and sugar on the other, or of mixtures, dissociation and other factors enter in.)

Kinds of Osmosis.—If pure water is separated from pure water by a water-permeable membrane, and if the temperature of the water on one side of the membrane is raised, the activity of the molecules on that side of the membrane will be increased. More of these molecules will therefore pass through the membrane. *Thermal osmosis* results.

If pure water is separated from pure water by a membrane permeable to water, and an electric potential difference is established between the two sides of the membrane, then there occurs a movement of water from the one (electropositive) side to the other (electronegative) side. This is *electroosmosis*, about which we shall have more to say later (page 358).

Again, we may have a so-called negative phenomenon which exists solely in virtue of the fact that our attention is centered on

one process while another is predominating; the former appears to be negative because the latter is the stronger and therefore positive. *Negative, or anomalous, osmosis* has been described as occurring through membranes with pores of different sizes, due to potentials set up. The potential difference set up between the two sides of the membrane causes, presumably, an anomalous flow of water, but it is anomalous only in regard to osmosis due to concentration; the electroosmosis taking place is in accordance with expectations. Ordinary osmosis, due to concentration, does not take place in one direction, because osmosis in the opposite direction, due to electric potential, is greater.

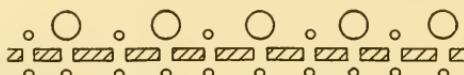


FIG. 103.—Molecules of pure water on one side, and of water and sugar on the other side of a semipermeable membrane.

Theory.—Why should water diffuse into a sugar or other solution against pressure? A number of theories have been advanced to answer this question. Let us imagine that 12 molecules of water are striking a membrane, which is permeable to water but not to sugar, along a given line on the pure-water side of the membrane. There will be fewer water molecules striking the membrane along a similar line on the other (solution) side, because sugar molecules are also there (Fig. 103). A certain proportion of the water molecules which strike the membrane will pass through from both sides. As there are more water molecules per unit volume on the pure-water side than on the solution side, more water molecules will enter the solution than will leave it. Consequently, there will be diffusion of water from the outside (pure water) to the inside (solution). This excess diffusion of water is osmosis.

The now generally accepted theory of osmotic pressure is that based on the fact that a solution is not in equilibrium with its solvent, at the same temperature and pressure. The two may be put into equilibrium by lowering the temperature of the solvent until the vapor pressure equals that of the solution, or one can change the pressure on either the solvent or the solution until equilibrium is established. The tension necessary to put the solution and solvent into equilibrium is the osmotic pressure. Such a comparison between osmotic pressure and vapor pressure

is based on the kinetic theory of molecular activity. This activity increases with rise in temperature. Molecules at the surface of hot water are moving about so actively that some escape into the air and form vapor. The hotter the water the more active are the molecules, and the more escape from the surface. The *escaping tendency* of molecules is a measure of the ease with which they leave the surface. It depends upon temperature and pressure. In order to escape from a liquid, molecules must overcome the surface tension, and energy is required to do this. Let us imagine two dishes, side by side, one filled with water and the other with a sugar solution. We may assume that there are at the surface of the water in the first dish 12 molecules over a given area. There cannot be so many water molecules over the same area of the sugar solution in the second dish, because sugar molecules occupy part of the space, just as in our previous example (Fig. 103). Here, the semipermeable "membrane" is the air between the two. It permits water molecules to enter and pass through it (evaporate) but not sugar molecules. As a result, there will be a greater vapor pressure above the surface of the pure water than over the sugar solution; in other words, a solution has a lower vapor pressure than its solvent; and, obviously, the greater the concentration of the solution the lower the vapor pressure. As the vapor pressure of a solvent (water) is greater than that of its solution (water and sugar), the solvent moves from a region where its escaping tendency (vapor pressure) is high to one where its escaping tendency is low. In time, the level of the solution in the one dish will rise, and the level of the pure water will fall. If pressure is applied to the surface of either, the corresponding vapor pressure will be increased. Osmotic pressure may then be redefined as the increase in pressure on a solution necessary to bring about equilibrium.

Methods of Determining Osmotic Pressure.—The direct method of measuring osmotic pressure is difficult if it is to be accurate. It is the one that was used by Pfeffer and consists in determining the weight of that column of mercury which is just sufficient to prevent osmosis. The osmotic pressure is then expressed in centimeters of mercury, or atmospheres (*e.g.*, a 0.1-*M* sugar solution has an osmotic pressure of $2\frac{1}{2}$ atmospheres). A manometer (pressure gauge) may be connected directly to the

osmometer, and the pressure read from the manometer scale. By such methods, Pfeffer obtained osmotic values for many concentrations of sugar.

Indirect methods of measuring osmotic pressure have to do with the depression of the freezing point, elevation of the boiling point, and lowering of the vapor pressure. Osmotic pressure is proportional to these properties. Vapor pressure varies inversely as the concentration of a solution while osmotic pressure is directly proportional to concentration; consequently, if the one is known, the other can be calculated. Indeed, we have defined the one, osmotic pressure, in terms of the other, vapor pressure.

Osmosis and the Theory of Solutions.—That solutions and gases are subject to the same laws was first set forth in the year 1885 by van't Hoff. The gas law of Boyle states that volume and pressure vary inversely if the temperature remains constant. The law of Gay-Lussac (or Charles) states that volume varies directly as temperature if pressure remains constant. From these two laws it follows that pressure varies directly as temperature if volume remains constant. The third gas law is the hypothesis of Avogadro. It states that equal volumes of all gases at the same pressure and temperature contain the same number of molecules. Our present purpose is to investigate these laws as applied to solutions in relation to osmotic pressure. The first gas law, therefore, may be reworded as follows: Osmotic pressure is proportional to concentration if the temperature remains constant. Qualitatively this has already been shown to be true in our visualization of osmosis. The more molecules of sugar there are, *i.e.*, the higher the concentration, the greater is the excess of inwardly diffusing water molecules over those diffusing outward. Quantitative proof is more convincing. At zero temperature ($0^{\circ}\text{C}.$) and atmospheric pressure (76 cm. of mercury), a gram-molecular weight or "mole," of a gas (*i.e.*, the molecular weight in grams, *e.g.*, 44 grams of CO_2) occupies a volume of 22.4 l. (this is true by experiment). If, as van't Hoff states, the pressure developed by a substance in solution is the same as that which it would exert if converted into a gas of the same volume and temperature, then one mole of a substance dissolved in 22.4 l. of water should give an osmotic pressure of 1 atmosphere. One mole (the molecular weight in grams) of every substance has the same number of molecules because, if a

small particle weighs 0.05 gram and a large particle weighs 0.25 gram, 5 grams of the former and 25 grams of the latter will give to each lot the same number of particles, *viz.*, 100. In the same way, 44 (the molecular weight) grams of carbon dioxide, CO_2 , and 342 (the molecular weight) grams of sugar, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$, will each have the same number of molecules. A mole of carbon dioxide (44 grams) and a mole of sugar (342 grams) having been obtained, it may then be observed whether or not the pressure of the gas is equal to that of the sugar solution, temperature and volume being alike in both instances. The pressure of 1 mole of a gas is 1 atmosphere when it occupies 22.4 l. at 0°C. If the volume (22.4 l.) is reduced to 1 l., then the pressure will be 22.4 atmospheres. If all our hypotheses are correct, then the same number of molecules of sugar in solution, *i.e.*, 1 mole of sugar, should give 22.4 atmospheres of osmotic pressure when dissolved in 1 l. of water. So much for theory.

Pfeffer found that a 4 per cent solution of cane sugar at 15°C. has an osmotic pressure of 208.2 cm. of mercury. This would be 197.4 cm. of mercury at 0°C. A 4 per cent solution (4 grams in 100 cc.) is the equivalent of 40 grams in a liter. A molar solution of sugar is $\frac{342}{40}$, or 8.55 times as concentrated; therefore, $10\%_{855}$ of the theoretical value of 22.4 atmospheres (22.4 atmospheres = 1,702.4 cm. of mercury) should equal Pfeffer's experimental value of 197.4 cm. of mercury for a 4 per cent solution. The theoretical value thus calculated is 199.1 cm. of mercury, a very close agreement to the experimental value of 197.4. (Since Pfeffer's day, results in still better agreement have been obtained.) The first law of gases, therefore, holds for solutions.

The analogy (first made by van't Hoff) is not a perfect one and does not hold accurately. It holds more perfectly for dilute solutions. Because of this, it is sometimes objected to, but the reply can be made that it is a striking and fundamental fact that a calculation such as that of Pfeffer, based on the theory of van't Hoff, should hold so very closely when tested experimentally. That the analogy is not perfect must be recognized. This is, however, everywhere true where the laws of one type of system are applied to another type. Gas pressure, solution pressure, and imbibition pressure take place in three distinct types of systems, yet they have certain properties in common. It has been shown that the imbibition pressure of a gel is closely com-

parable to the osmotic pressure of a solution (page 204). The deviation between vapor pressure and osmotic pressure is seen in the mathematical expression of the two— $PV = RT$ for the gas law and $PV = KT$ for the osmotic law. The two are nearly the same but not quite, because R is not the proper constant for water vapor. The systems are not identical; there are variables of which we are ignorant; but the analogy is a very close one and indicates a fundamental similarity. How close it is is not always fully appreciated. Frazer has shown that with a substance such as mannite, where there is little heat of solution, the agreement of the gas laws is quite surprising, even up to as high as 20 atmospheres pressures, and there are many gases which begin to diverge from the so-called gas laws at or before pressures of this magnitude are reached.

Physicochemical Applications.—An interesting application of osmosis to nonliving physical and chemical processes is that of deVries. Chemists were considering the probable formula of the sugar raffinose, a trisaccharose found in molasses. There were three possibilities: $C_{12}H_{22}O_{11} \cdot 3H_2O$, $C_{18}H_{32}O_{16} \cdot 5H_2O$, and $C_{36}H_{64}O_{32}$. Sucrose and raffinose may be compared by balancing them on opposite sides of a membrane, then, when the two are in osmotic equilibrium, each solution will contain the same number of molecules per unit volume. This is true of all (nonionizable) isotonic solutions. The total weight of the raffinose (*i.e.*, the concentration) which is isotonic with sucrose being known, it is an easy matter to calculate the weight of one raffinose molecule from the following simple proportion:

$$\frac{\text{Total weight of sucrose}}{\text{Weight of one sucrose molecule}} = \frac{\text{total weight of raffinose}}{\text{weight of one raffinose molecule}}$$

If a sucrose solution of 3.42 per cent is chosen, 342 being the molecular weight of cane sugar, then the calculations are simplified. That concentration of raffinose which is isotonic with 3.42 per cent sucrose is 5.96 per cent. The relationship then becomes

$$\frac{3.42}{342} = \frac{5.96}{M}$$

(M being the desired molecular weight of raffinose); therefore, $3.42 M = 342 \times 5.96$, and $M = 596$, the molecular weight of

raffinose. The three possibilities of the chemical constitution of raffinose as given by the chemists being now considered, one of these may be observed to have a molecular weight of 594, *viz.*, the second formula, $C_{18}H_{32}O_{16} \cdot 5H_2O$, the slight difference of 2 being due to experimental error.

A botanist like deVries would be more interested in making the plant cell serve as his osmotic system rather than setting up an apparatus in the laboratory. Furthermore, deVries, in using plant cells, could perform the entire experiment before a physical chemist could get his apparatus assembled. DeVries first found that concentration of sucrose which was isotonic with cells; then he did the same for raffinose. The two sugars, being isotonic with the same osmotic system (that of the cells), were obviously isotonic with each other and could then be compared.

Here and there on the foregoing pages care has been taken to emphasize the fact that comparison between the concentrations of solutions from the point of view of their osmotic behavior can be made only of nonionizable substances such as sugars. It is obvious that if equimolecular solutions have the same osmotic pressure, this can be true only if the substances stay not only equimolecular but of equal particle number, whatever these particles may be. Consequently, if a solution of sodium chloride is equimolecular with one of sugar, it will not be of the same osmotic pressure if each salt molecule breaks down (dissociates) into two particles, or ions. Each ion will possess osmotic activity just as does a molecule; therefore, if dissociation is complete, a monovalent ionized salt should have twice the osmotic pressure as the unionized sugar of equimolecular concentration. This is true except for the discrepancy introduced by the fact that the salt either does not dissociate fully or some other factor enters in which makes it appear that the salt has not fully dissociated (page 298). That monovalent salts, each molecule of which dissociates into two ions, do not have twice the osmotic pressure of equimolecular concentrations of sugars, and that divalent salts, with their three ions, do not have thrice the osmotic activity of sugar led Arrhenius to postulate the incomplete dissociation of electrolytes.

The Plant Cell as an Osmotic System.—Plant cells usually contain a large central vacuole or cavity filled with a solution. The vacuole and the surrounding protoplasm are each enclosed

by a membrane (Fig. 2). Both membranes are of protoplasmic material and function osmotically; *i.e.*, they are semipermeable. Within the vacuole is a solution of salts, carbohydrates, proteins, etc., certain ingredients of which cannot readily pass through the membrane. The vacuole is, therefore, an osmotic system, a solution of relatively high concentration within a selectively permeable membrane surrounded by a solution of low concentration. The outer solution, in the case of an aquatic plant, is the water of the pond or, in the case of the root hairs of terrestrial plants, the soil water; if the cell is part of inner tissue,

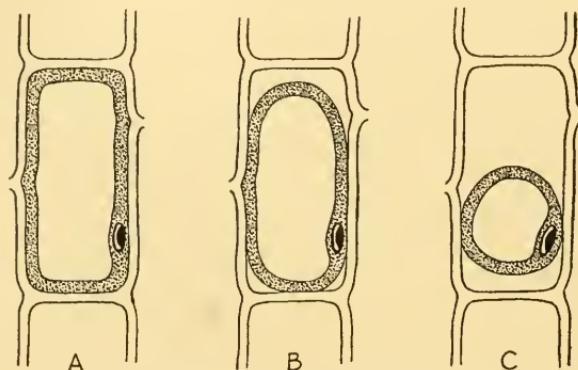


FIG. 104.—Stages in the plasmolysis of a plant cell.

then the plant juices or intercellular sap constitute the surrounding solution.

Water in an osmotic system moves in excess toward the solution of higher concentration. If the concentrations are reversed, the movement will be reversed, and any pressure already existing on the one side will be released. This holds true for the plant cell. If the osmotically active substances dissolved in the water of the vacuole are the equivalent of, say, 3 per cent of common salt, then, if the cell is bathed in 5 per cent salt, osmosis will be reversed, and the turgor in the cell relieved, so much so that the cell (protoplast) may shrink away from the (relatively) rigid cellulose wall (Fig. 104). This shrinking is known as *plasmolysis*. Naturally, if the inner and outer solutions are equivalent (in osmotic activity), there will be no movement of water in either direction and no change in pressure. If plant tissue, such as the leaf of Elodea, is put in salt or sugar solutions of different concentrations, varying from 1 to 10 per cent of salt (potassium

chloride) or 5 to 20 per cent of sugar (sucrose), the cells that are in 1 or 2 per cent salt or 5 to 8 per cent sugar will show no change, while those in 3 per cent salt may, and those in 4 per cent are certain to, show a slight shrinking away from the walls within a few minutes. Those in 5 or 6 per cent salt will show a pronounced shrinking away (*B*, Fig. 104), while those in 10 per cent salt (or 20 per cent sugar) will, in a few minutes look like spheres within boxes much too large for them (*C*, Fig. 104). The protoplasts have shrunk owing to reduction in turgor (but *not* in osmotic pressure; this has *increased*, owing to increase in concentration of sap from loss of water).

A solution that has the same osmotic pressure as has the cell sap (in the preceding experiment, this was true of 3 per cent potassium chloride) is isosmotic or isotonic with the cell sap. As we have said, solutions of lower concentration are hypotonic, and those of higher concentration are hypertonic. The last named alone cause plasmolysis. The concentration of an isotonic solution (one which just does not cause plasmolysis) is known as the *critical plasmolytic concentration*. The osmotic pressure of a salt or sugar solution which is of a critical plasmolytic concentration is the osmotic pressure of the contents of the cell vacuole, for the two are isosmotic. In this way is the osmotic pressure of plant cell sap determined. It tells nothing of the true pressure or turgor within the cell. The turgor within plant cells differs greatly. That this is true is likely from the great differences in osmotic pressures of cell saps. A common average is 10 atmospheres. The osmotic pressure of the cell sap of molds (*Penicillium*) which grow on very concentrated solutions or in the fumes of highly concentrated acid may reach maximum values of over 100 atmospheres. This must be true if the living plant is to draw water from solutions of high concentration, *i.e.*, of low water content. But this is osmotic pressure and not turgor. The actual pressure within such cells need not be great. In fresh and crisp plants, turgor is high; in wilted plants, it is very low, but the osmotic pressure may not vary in the two cases; if the same plant is involved, the osmotic pressure will be greater in the wilted plant of lower turgor. (Loss of water means higher concentration and therefore higher osmotic pressure of the cell sap.) High osmotic pressure suggests high turgor but is no proof of it and is certainly not a measure of it.



The Animal Cell as an Osmotic System.—Osmosis was originally, and has long remained, a botanical problem, though it was soon taken up by the chemists. It has never been a leading question in animal physiology, but it has been studied there by Lucké and others. What little turgidity animal cells possess (animal tissues are usually flabby) has been attributed to imbibition pressure, such as makes a block of swollen gelatin firm, rather than to osmotic pressure (or turgor). Nevertheless, Lucké measures what he terms the turgor of animal cells (eggs of echinoderms and red blood cells) and finds them to be perfect osmometers, obeying the law of Avogadro (the law of equimolecular concentration).

The Role of Osmosis in Life.—Osmosis maintains the turgidity or firmness of plant tissue and thus keeps the plant supplied with water for metabolic processes. When turgor is normal, plant tissue is firm. When the water supply is lowered and turgor reduced, the tissue becomes flabby, and the leaves droop. This is wilting. The amount of water which plant tissue can hold is strikingly illustrated in the succulents—the extremely juicy plants. These have spongy stems, like cacti, or fleshy leaves, like those of *Sedum*, which are firm and “plump” with water retained under pressure. Barrel cacti are cut by Indians to obtain water for drinking.

Osmosis contributes to the maintenance of the water supply in plants through the so-called transpiration stream. The upward flow of sap against gravity in trees is one of the great problems in botany. There is apparently an osmotic gradient in plants, with highest osmotic pressure in the leaves and lowest in the roots. But if the flow of sap is by osmosis, it must be through living cells, as only they, with their semipermeable protoplasmic membranes and vacuoles, are osmotic systems. Actually, the sap appears to flow through dead wood (xylem) most of the way. Water does, however, first enter the plant through living cells (root hairs), so that at least the first movement of water into the plant is a purely osmotic phenomenon. Water continues to diffuse from the soil into the living plant cell as long as the percent of soil moisture is sufficient to keep the concentration of the soil solutions hypotonic to (of a concentration less than that of) the concentration of salts and sugars in the cell vacuole.

The turgor of plant cells does not remain constant. The concentration of the cell sap (therefore its osmotic pressure) and the permeability of the protoplasmic (or vacuolar) membrane undergo changes from time to time which appear to be capable of "control" by the cell or by the plant as a whole. This is probably true in the diurnal (day-and-night) and other movements of leaves and flowers, where osmosis may be the operating mechanism. An interesting case of the movement of leaves is the closing of the leaflets of the sensitive plant, *Mimosa pudica*. This plant closes its leaflets and drops its petiole when irritated by a mechanical disturbance such as cutting or mere touching. If the leaflet at the tip of a leaf is disturbed, it immediately closes by folding close to the stem, and a moment later its mate does likewise. Very soon, the adjoining leaflets close, then those farther down the petiole, until all are folded. The stimulus continues down the leaf stem until it reaches the base, where there is a swelling called the *pulvinus*. The petiole then falls until it hangs close to the main stem of the plant. The stimulus does not stop at the base of the irritated leaf but turns up and down the main stem to other leaves the leaflets of which close in the reverse order from that of the first leaf. The manner in which this stimulus is transmitted is one problem, but here we are concerned with the mechanism closing the leaflets and dropping the petiole. If the turgidity of the cells of plants is reduced, the tissue collapses. When the pulvinus is rigid with water, it holds the petiole and leaf up; but when pressure is released, the tissue of the pulvinus becomes soft and collapses, and the petiole falls.

Plants grown experimentally in water cultures thrive best when the salt solution is not only of the correct chemical constitution but also of the correct osmotic value. A solution hypertonic to the sap will cause the cells to lose water; if too strong, the cells may become plasmolyzed, resulting in injury and death. A hypotonic solution is the natural one in the soil; plants may then take in water rapidly. Variation in tonicity (osmotic pressure) of soil water acts as a stimulus, and plants then do better. Such changes constantly occur in the soil, owing chiefly to rain.

Marine organisms are bathed in an aqueous medium which is of very constant composition. (Some variations and changes

exist.) The same is true of cells within the bodies of plants and animals. Body fluids are aqueous solutions the salt content of which is kept fairly constant. These facts indicate that the osmotic pressure of a solution is of considerable significance for a plant or animal, even though some other facts show that osmotic pressure has no marked influence—certainly little in comparison with the chemical constitution of the solution; thus, animal cells grown in tissue culture thrive equally well in culture solutions of different osmotic values. Experiments by Jacques Loeb on the parthenogenetic development of eggs offset these in tissue culture.

Parthenogenesis is the growth of an egg without fertilization, *i.e.*, without a sperm having entered. What actually starts the egg dividing in parthenogenetic development it is difficult to say. Loeb was uncertain about it, but he laid emphasis on one of two factors—osmotic pressure and the formation of a fertilization membrane. (Such a membrane is formed at the time of fertilization and becomes a conspicuous structure, considerably larger than the egg which it surrounds.) It was possible to cause sea-urchin eggs to develop into larvae by simply exposing them for two hours to hypertonic sea water, *i.e.*, sea water to which salt or sugar had been added. Later experiments showed that other factors may also bring on or hasten parthenogenetic development; hydroxyl or potassium ions and acids will do so. But unfertilized eggs can be activated without these substances and without membrane formation simply by adding a nonelectrolyte such as sugar to the sea water, *i.e.*, by hypertonic water. It thus appears that osmotic pressure is the primary stimulus of parthenogenesis when occasioned by solutions.

The entrance of water into the large vacuole of plant cells is a purely osmotic process. In one-celled animals, and in some few plants in a modified form, there exists a contractile vacuole which pulsates rhythmically, discharging its contents to the outside and then refilling again with liquid from within (Fig. 25). Such contractile vacuoles are especially characteristic of the fresh-water Protozoa. Usually there is but 1, though certain amoebae may have as many as 10. Their function appears to be that of ridding the unicellular organism of waste organic matter and excess water taken in in the process of feeding. The

water engulfed with food is held for a time in food vacuoles, later diffusing into the protoplasm and ultimately finding its way into the contractile vacuole. This vacuole can, therefore, be compared with the kidney of higher organisms. Other types of smaller vacuoles may serve a similar purpose.

The contractile vacuole may fill by osmosis, but the mechanism of contraction (emptying) is not known. Furthermore, for continued operation, the concentration within must remain higher than that without, yet, as the vacuole enlarges, during diastole, its contents become diluted. If a contractile vacuole fills by osmosis, then why at a definite point does it suddenly empty itself? We do not know. Syneresis (page 146) or electroendosmosis (page 358) or membrane equilibria (page 203) may be involved.

The role of osmosis is prominent within the animal body and constitutes an important study in medicine, as is evident from the work of R. Höber on the kidney and other adenoid (gland-like) tissues. The osmotic pressure of the blood is 7 atmospheres. The extent to which water will enter or leave the blood stream is in great measure determined by this value. The pH (acidity) of the blood is 7.38. The pH of the gastric juice is from 1 to 2. Osmotic work must be done in concentrating H^+ ions in the gastric juice to the extent of a million times that in the blood stream.

The kidney is an osmotic machine, as are also the sweat glands of animals and the water-secreting glands (hydathodes) of plants. The functioning of the kidney, like that of most glands (*e.g.*, the nectaries of flowers), may be primarily osmotic, but other forces are certainly involved, for a diffusion takes place against a concentration gradient. The concentration of urea in urine is sixty times that in the blood. The mechanism responsible for this cannot be solely osmotic; certain other forces (again possibly electroendosmosis) must be active.

CHAPTER XII

IMBIBITION

Protoplasm takes in water by *imbibition*. In general, imbibition is the taking in of water and consequent swelling by gels. Most organic gels, such as cellulose, agar, gelatin, and starch, swell when in water. They are, therefore, known as jellies, in distinction from coagula. Gels (jellies and coagula) usually possess high elasticity in the true physical meaning of the word (an extreme case is that of glass), but extensibility, or capacity to be stretched, is primarily a property of jellies. Extensibility and imbibition thus distinguish jellies from coagula. *Turgescence* is a term commonly used to denote swelling through the taking in of water. Inorganic gels, such as pumice stone and silica gel, which do not swell, are nonturgescent. Such gels may take up water even more readily than do jellies, but they do not exhibit that one indissociable property of imbibition, *viz.*, swelling, and its inevitable companion imbibition pressure. (Solvation and hydration are sometimes used as synonyms of imbibition.)

Imbibition pressure is a familiar expression in biology and colloidal chemistry. The volumetric increase due to the swelling of jellies is great, and the magnitude of the pressures developed may be enormous. The considerable increase in volume of a gel, due to swelling, is illustrated in the case of the stalk of the seaweed *Laminaria*, which will swell several hundred per cent when soaked in water after drying. Starch, in taking up water, may develop a pressure of several thousand pounds per square inch. The stupendous pressure developed by imbibition is illustrated in the use of wood by the Egyptians to dislodge huge blocks of stone.

A more delicate operation is that involving the slow separation of the bones of a skull by the swelling pressure of wetted peas filling the skull. The cracks in a boat or in a wooden bucket are closed by the imbibition pressure of wood. Wooden parts of certain machinery must be kept permanently wet for use. The

imbibition pressure involved in the swelling of seeds causes bursting of the seed coat, thus allowing the embryo to emerge.

Gels may hold an extraordinary amount of water by imbibition. Some colloidal solutions (of cadmium and of calcium germanate) may set to a firm gel even though the concentration of solid matter is but 0.2 per cent, and the water content, therefore, 99.8 per cent. This is almost equaled by the jellyfish, which contains 99 per cent water.

The shrinking of gels from loss of water produces a tension, or pull, as great as imbibition pressure. This is illustrated by gelatin, which, when shrinking, pulls with sufficient force to chip the glass to which it is attached.

The physical nature of imbibition is little understood. The problem is best handled by approaching it along those paths which will give the surest footing; then the venture may be taken a little way into the field of uncertainty. Capillary forces (surface tension and adhesion) are responsible for the absorption of water by porous gels, such as the inorganic silica gel. These nonextensile gels contain myriads of pores or capillaries. Such a structure of the silica gel and the nature of the force filling it with water are questioned by none. The sizes of the pores have been accurately determined, with a minimum of $5\text{ m}\mu$, which gives to the gel an ultramicroscopic spongelike structure (Fig. 90). Pumice stone is a similar example; water enters its pores under the pull of surface forces acting in tiny spaces. Pumice stone and silica gel are of the nonswelling type. We do not, therefore, have to do with imbibition in them.

The turgescent gels, or jellies, are chiefly organic, gelatin and albumin being typical examples. Some are organic salts; among these are carbohydrates, such as starch, cellulose, and agar, and certain soaps and dyes, which form perfect jellies. Agar is a trisaccharide obtained from seaweed and forms a typical jelly much like gelatin in its physical properties, though its chemical nature is quite different. Concerning the structure of these jellies almost nothing is known, with the exception of cellulose.

The mechanics of swelling is apparently not due to capillary forces, which one might assume to be operative in the case of porous material such as wood (cellulose); capillarity is not a distending force; it is a tension. The pull of a surface film which

will draw a free droplet of water into a sphere is not going to force the structural framework of cellulose apart.

There are other difficulties in interpreting the swelling of jellies on the basis of capillary forces. To what is due the further swelling of a jelly which, having swollen to its maximum capacity in pure water, swells more when acid is added? There are no capillary spaces left to account for the additional swelling, and those already full have become larger through swelling, which *lessens* capillary attraction. When wood swells, the filling of tiny spaces with water by capillarity (surface tension) is the first force which comes into play, but the further swelling of the wood is due to forces other than capillary ones. Another objection to capillarity as the mechanism underlying swelling in jellies lies in the fact that there is no conclusive evidence of a porous structure in jellies (*e.g.*, gelatin). No capillaries are visible in a gelatin jelly, and there is doubt as to the likelihood of there being ultramicroscopic capillaries. If the structural unit of gelatin is a micelle, then the jelly structure is that of a sponge, and the spaces are capillaries; but if the structural unit is a molecule, as some believe, then capillaries, as ordinarily thought of, do not exist. With molecular dimensions come other forces. Among these is adsorption.

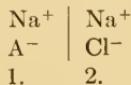
Water molecules orient themselves at the surface of some substances very readily. Such adsorbed water is known as water of hydration. The older and still prevalent idea of the nature of water of hydration (or crystallization) is that the bond between the water and the crystal molecules is one of secondary valence or adsorption, which implies that the union between the water and the crystal molecules is a loose one, as the two are readily separable, *e.g.*, by heat. The newer viewpoint sees no special, clear identity of the water molecule. The crystal with the water is a new compound altogether, with a new arrangement of the atoms.

Imbibition vs. Osmosis.—The swelling of gelatin has been interpreted as an osmotic phenomenon, the claim being made that imbibition and osmosis are identical in so far as the forces involved are concerned. Thus, J. Loeb said, "the reason that osmotic pressure, the viscosity of protein solutions, and the swelling of protein gels are all influenced in a similar way by electrolytes is that all three properties are in the last analysis

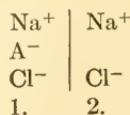
functions of one and the same property, namely, osmotic pressure." This may be true, and an attempt will be made to show why some think that it is; but there are those who regard imbibition as a process quite distinct from osmosis. The latter term has heretofore always been applied to the forces involved in a system where a membrane encloses a solution. Freundlich states emphatically that it is fundamentally false to regard osmotic pressure as identical with imbibition pressure. There is heat of imbibition, but no heat of osmosis. (This may be merely a matter of degree.) More significant are the facts that osmotic pressure is proportional to concentration, while imbibition pressure is proportional to a high power of the concentration.

Botanists, concerned as they are with the swelling pressure of gels (cellulose, starch, and agar), on the one hand, and the turgor of the cell vacuole, on the other, regard the two as distinct because the respective systems in which they take place are mechanically, *i.e.*, structurally, different.

The Donnan Equilibrium.—We shall have occasion a number of times to refer to what is known as the *Donnan equilibrium*. Here it serves us as an interpretation of imbibition. The mathematical analysis of a simple osmotic equilibrium by Willard Gibbs, who stated that the chemical potential of a solution is of the same value on both sides of a differentially permeable membrane, and the suggestion of Wilhelm Ostwald that significant relationships must exist between ions when a selectively permeable membrane separates two solutions served F. G. Donnan of London as a basis for his theory of membrane equilibria. In its simplest form, the Donnan equilibrium is as follows: If a membrane separates a solution of sodium chloride from one of sodium albuminate, then there will be the following distribution of ions at the beginning of the experiment (the albumin anion is represented by A^-):



After osmosis has been allowed to act, the Cl^- ion on the one side will have diffused to a greater or lesser extent to the other side. As the albumin ion A^- cannot pass through the membrane, the following condition will exist at the end of the experiment:



For thermodynamical reasons, it was known that the product of the concentration of the diffusible ions of a pair on one side of the membrane equals the product of these same ions on the other side, or

$$[\text{Na}^+]_2 \times [\text{Cl}^-]_2 = [\text{Na}^+]_1 \times [\text{Cl}^-]_1$$

(Brackets signify molar concentrations.) This equation expresses mathematically the equilibrium that bears Donnan's name. The law was known (it is the basis of much modern physical chemistry); Donnan applied it to the situation involving a membrane. Were the membrane permeable to all ions, the law of diffusion would demand that the sum of the ions on the one side of the membrane should equal that on the other, but this cannot be true because of the inability of the large albumin ion to penetrate the membrane. There are thus set up two forces, or pressures, opposing each other—osmotic, demanding equal sums of ions; and electrical, demanding equal products of ions, on the two sides of the membrane. Obviously, equal sums and equal products cannot exist simultaneously. Donnan assumed that at equilibrium electrical forces dominate when $[\text{Na}]_1^+ > [\text{Na}]_2^+$ and $[\text{Cl}]_1^- > [\text{Cl}]_2^-$. That this is actually the case may not necessarily (experimentally) be true; for example, were sugar added to one side of the membrane, the osmotic pressure thus introduced would certainly overcome the electrical pressure and change the ratio of the concentrations on the two sides of the membrane. In any case, there is, at equilibrium, a balance between osmotic and electrical forces, which presumably leaves an excess osmotic pressure on one side of the membrane.

If instead of a membrane separating an albumin solution from a salt solution, a block of gelatin is immersed in a salt solution, the same conditions exist, except that the force of cohesion between the gelatin ions of the solid block is substituted for the membrane. The osmotic pressure developed causes swelling of the gelatin. According to Proctor and Wilson gelatin immersed in hydrochloric acid forms gelatin chloride, which ionizes into gelatin cations and chlorine anions. (The situation is the same as

in the case of albumin immersed in a sodium chloride solution.) The same unequal distribution of ions results, with an imbibition or osmotic pressure within the gelatin.

The application of the Donnan equilibrium to experimental (not theoretical) imbibition conditions has met with some objections. The ratio of the concentration of the diffusible ions appears to be an assumption. So also is the statement that a salt of gelatin is formed when the latter is in an acid or alkaline medium. If a salt is formed and if its formation is a necessary prerequisite of the equilibrium and consequent swelling, we then have *no interpretation of the swelling of gelatin in pure water* or of the effect of salts on the degree of swelling of isoelectric gelatin. The Donnan membrane equilibrium, sound when applied to systems where its demands are met, does not interpret all phases of the general problem of the swelling of gels.

In spite of doubts and difficulties, the concept of membrane equilibrium offers interesting suggestions for a possible mechanism of a number of biological processes. A high concentration of the nondiffusible ion A^- will inhibit diffusion of the sodium chloride to such an extent that if sodium chloride is added to the side containing the nondiffusible ion, there may be diffusion to the other side against an opposing osmotic pressure—in other words, *excretion* of sodium. It is possible that glands may function in this manner and thus be able, as they are, to excrete against concentration gradients (see also page 378).

The problem of the identity of imbibition and osmosis is one that cannot yet be solved. While it still seems desirable to distinguish them, certain criticisms are not fully justified. It is stated that imbibition pressure should not be confused with osmotic pressure, because the former may reach values greatly in excess of the latter. Actually, both are infinite. There is no force that can keep dry sugar from becoming wet. The same is true of dry gelatin. No force could keep it from absorbing some water.

Plants sometimes make use of one method, osmosis, and sometimes of the other, imbibition, for getting water. Gortner cites the case of seeds which take up water from a saturated lithium chloride solution (osmotic pressure, 1,000 atmospheres), although their salt content is sufficient to account for only a few atmospheres of osmotic pressure. Cacti with osmotic pressures not in

excess of 6 or 7 atmospheres take up water from very dry soil. Newton put cactus stems in a desiccator with sulphuric acid for six months. In this atmosphere of nearly zero humidity, they lost less than 10 per cent of their water. It is assumed that imbibition forces held the water. Again it is impossible to say with any degree of certainty whether osmotic or imbibition forces are responsible.

The following experiment is an interesting one in which osmotic forces are pitted against imbibition ones: If a series of cubes of a gel (say, 25 per cent gelatin), all of one size, are placed in sugar solutions of different strengths, water passes from the gel to the



FIG. 105.—Cubes of gelatin placed in solutions of sugar of different concentrations. Left, low concentration; right, high concentration.

solution, or vice versa, according to the concentration of the surrounding sugar (Fig. 105). One cube in the series may maintain its original size; the others become larger because of the greater imbibition pressure of the gel, or smaller because of the greater osmotic pressure of the surrounding solutions.

The imbibition-*vs.*-osmosis controversy when applied to protoplasm brings up an old discussion on the miscibility of protoplasm in water.

Imbibition and Solution.—In attempting to distinguish between osmosis and imbibition as the mechanism by means of which protoplasm takes up water, and therefore at the same time deciding whether protoplasm is miscible or immiscible in water, we may simply be again involving ourselves in a futile polemic similar to the attempt to distinguish between osmosis and imbibition. There is, however, one very important and fundamental question involved in the distinction between imbibition and solution; it is a question of structure. All agree that protoplasm takes up water with avidity; but does it do so osmotically,

in the sense of going into solution, or by imbibition, in the sense of soaking it up, as does a jelly or a blotter? The problem is one that emphasizes the vital importance of structural continuity in protoplasm. Sugar, not blotting paper, is miscible in water. Imbibition and solution both depend on the preferential adsorption of water by molecules, but the actual mechanism of the two phenomena appears to differ. The molecules of soluble substances are free when in solution (as is sugar in water). Those of substances that imbibe water are held together (as in a gelatin block). There is no limit to the amount of water in which a miscible substance (sugar) will diffuse; but a substance (gelatin) that imbibes water and is immiscible in it takes up a definite maximum amount and then stops. Structural continuity holds it together. This is the case with protoplasm; it imbibes water but does not go into solution with it.

Dujardin, one of the earliest workers on protoplasm, described protoplasm as a living jelly, *insoluble* in water. Hertwig referred to it as a viscous, *water-immiscible* substance; and Butschli said the same, *viz.*, that protoplasm is not miscible in water. There are, however, supporters of the opposite opinion. They base their view on the rapid diffusion of water when it is injected into protoplasm. The same would be true if water were injected into a moist, but not saturated, sponge or blotter. There is much evidence to show that protoplasm possesses a continuous framework, comparable to a "brush heap," not fixed or rigid but labile. This framework exhibits great affinity for water but is immiscible in it, just as is true of gelatin. Much of the inorganic and organic matter that permeates the protoplasmic framework is in true aqueous solution.

Biological Applications.—The manner in which protoplasm takes up water is but one of the many biological applications of imbibition. The following are others.

Growth, in so far as it involves mere distention, or getting big, and germination, which is a form of growth, are due in part to imbibition.

Muscle action has been regarded as due to swelling or imbibition, though the evidence for it is not sufficiently convincing. It is said that a bee's wing may vibrate two hundred times a second. Hofmeister believed that protein strands as fine as the fibrils in the muscle of a bee's wing may imbibe water and give

it up again (syneresis) rapidly enough to permit contraction and relaxation two hundred times a second. Gortner states that a contracted muscle has a higher water content than has a relaxed muscle.

Aging may be a matter of drying up—the reverse of imbibition—but whether we get old because we dry up or dry up because we get old is a question. In any case, it appears that the capacity of our protoplasm to hold water decreases with age. Růžička attributes aging to a less hydrated condition of the protoplasm, due to the gradual going over of albumin into globulin, which albumin does spontaneously.

Body form, in certain instances, may be due to dehydration—the reverse of imbibition. Hatschek suggests that the gastrulation of an embryo takes place in the same manner and for the same reason as does the invagination of a two-thirds segment of a sphere of gelatin on drying.

CHAPTER XIII

VISCOSITY

The viscosity of protoplasm is intimately associated with vital processes. Growth, reproduction, mitosis, amoeboid movement, streaming, permeability, adsorption, metabolism, and other cell functions all involve, and therefore are in part determined by, the viscous state of protoplasm. Change is as typical of the viscosity of protoplasm as it is of life as a whole. Change in protoplasmic consistency may range from the extreme fluidity of a thin liquid to the firmness of a rigid jelly. The viscosity of any one region in a cell may undergo pronounced changes with change in physiological activities. Different regions in a cell may also differ greatly in viscosity at any one time. To fail to appreciate these facts is to fail to have a clear understanding of protoplasmic behavior and the physiology of the cell. A viscosity value of protoplasm is all but meaningless unless the species, the kind of tissue, the physiological state, and the precise region in the cell are specified.

Dujardin, who with von Mohl first studied protoplasm intensively, characterized it as a "living jelly," a "glutinous substance"; and von Mohl described it as a "viscid mass." Thus did both lay emphasis on the high consistency of the living substance.

Terminology.—Viscosity is that property of liquids which causes them to resist flow. In the mind of the layman, it is more characteristic of thick liquids such as molasses and pitch than of thin ones such as water, though actually it is possessed by all substances that flow, including gases. Viscosity is practically synonymous with *consistency* and is the reciprocal of *fluidity*. The importance of viscosity in the manufacture and use of paints, lacquers, paper, rubber, cellulose, porcelain, and many other types of plastic material has led to the naming of the study of viscosity and thus establishing it as a distinct branch of physical chemistry, *viz.*, *rheology*—the science of flow.

Methods.—Viscosity values may be *specific*, that is to say, *relative* to a standard; or *absolute*. In the first case, water is the customary standard and is taken as unity (at 0°C.). In the second, the values are expressed in dyne seconds per square centimeter, or *poises*. The absolute value is known as the coefficient of viscosity, with η as its symbol. The relative viscosity value of glycerin is 830, which means that glycerin is 830 times as viscous as water (at 20°C.). Several absolute values or coefficients of viscosity are given in the following list:

Water.....	0.018 at 0°C.
Water.....	0.01 at 20°C.
Olive oil.....	0.84 at 20°C.
Glycerin.....	8.30 at 20°C.
Glucose.....	27,000.00 at 67°C.

One may express the values in centipoises (1 centipoise is one one hundredth of a poise). Inasmuch as the absolute viscosity of water in centipoises at 20°C. is 1.005, then viscosity values determined relative to water become absolute values in centipoises.

An instrument for measuring viscosity is known as a *viscosimeter*, or *viscometer*. There are numerous types, the simplest being a glass capillary tube through which the liquid is allowed to flow. The rate of flow is determined by a number of factors, such as the fineness of the capillary and, naturally, the viscosity of the liquid. These factors are all combined in a formula developed by Poiseuille which will be considered shortly.

In the nonliving world, ways of measuring viscosity involve most often a capillary viscometer in which the rate of flow of the liquid through a fine capillary is compared to that of a standard solution, usually water. The torsion method (with a revolving drum) of Couette is frequently used in the laboratory. Commercially, the gravity method, in which balls are allowed to fall or air bubbles allowed to rise in the unknown and standard liquids, is common practice. But of greater interest to us are ways of measuring protoplasmic consistency. Should the physiologist find the methods crude and the values inexact, let him remember that it is *living* matter which is being investigated, that it must *remain alive*, and that it can usually be had only in microscopic droplets. The rheologist, in particular, should also

remember that protoplasm, in addition to being alive, offers all of the problems of the anomalous flow of an elastic liquid.

Attempts to measure the viscous property of protoplasm with precision are mostly of relatively recent date. A pioneer endeavor to measure a closely related property is that of Pfeffer. He was able, by attaching minute weights to living protoplasmic threads, to determine the load that a strand of myxomycete plasmodium would support. What was measured was the tensile

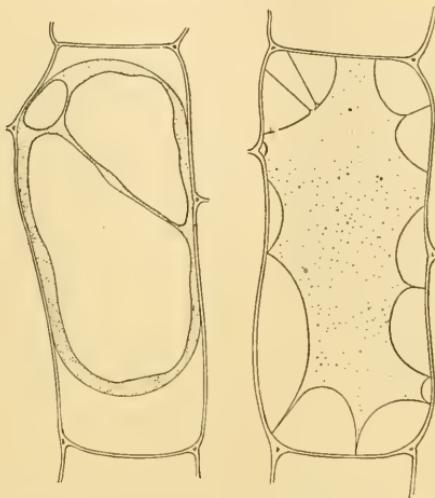


FIG. 106.—A plasmolyzed plant cell with rounded protoplasmic surface (left), and one with concave surface and protoplasmic strands (right).

strength of protoplasm, or, as Pfeffer expressed it, the cohesive force.

The Plasmolytic Method.—Under this caption, several methods bearing directly or indirectly on contractility will be considered.

The configuration of protoplasts (the cell contents as a whole) after plasmolysis is thought to be an indicator of protoplasmic viscosity. The average plant cell when plasmolyzed (in a strong or hypertonic sugar solution) has a smooth, convex surface, with little or no adhesion of the protoplasm to the cellulose wall (Fig. 106). This presumably indicates low viscosity. At times, a concave, irregular form, often with many fine attached protoplasmic strands, is obtained; this condition is said to be maintained as a result of high consistency (Fig. 106). But often convex plasmolyzed protoplasts are obtained with many

strands attached to the cell wall. This suggests that the concavity and the numerous and persistent threads are more likely due to greater tensile strength and greater adhesive qualities of the protoplasm rather than to higher consistency.

The Gravity Method.—A common commercial method for determining the viscosity of liquids is that of allowing lead shot to fall through a long column of the liquid. The time of fall is taken and compared with that obtained in a standard liquid such as water. Timing the rise of lighter bodies such as air bubbles does just as well. The viscosity value obtained in this way may be relative to water or absolute in poises when it is calculated with the aid of Stokes' law of falling bodies, which is expressed by the formula

$$V = \frac{2r^2(D - d)g}{9\eta}$$

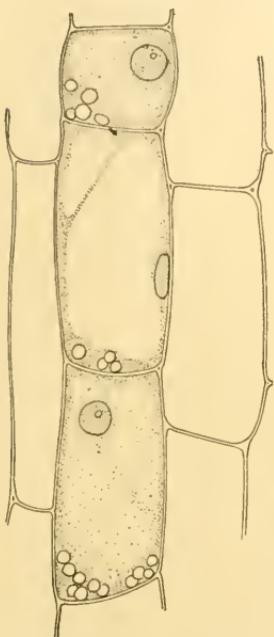


FIG. 107.—Statoliths (starch grains) resting at the bottom of plant cells.

where V is the velocity of fall; r , the radius of the particle; D , the specific gravity of the particle; d , the specific gravity of the medium; g , the gravity constant; and η , the viscosity of the medium.

Němec and Haberlandt used a similar method for measuring the consistency of protoplasm. The living cell is itself made to serve as a viscometer. Plants sometimes contain freely suspended starch grains (statoliths), which normally lie at the bottom of the cell and presumably serve as gravitational sense organs (Fig. 107). If the cell is turned upside down, the starch grains slowly fall. By exposing a living cell to view, repeatedly reversing the plant, and noting the time of fall of the grains, an indication of the consistency of the protoplasm can be obtained. The viscosity value is calculated with the aid of Stokes' law.

The Centrifuge Method.—Particles suspended in a liquid can be thrown out by centrifuging if they are of a density different from that of the medium. The centrifugal force required

to displace them will vary directly with the viscosity of the liquid.

The eggs of fish or echinoderms when centrifuged show stratification of the protoplasmic granules (Fig. 108). The number of turns of the centrifuge handle per unit of time required to stratify the egg granules is a measure of the comparative viscosity of the protoplasm. Values are calculated with the aid of Stokes' formula. Runnström finds the viscosity of the echinoderm egg to be eight hundred times that of water as determined by centrifuging; this is almost exactly the viscosity of glycerin. Much lower and higher values have been obtained by other workers using the same method on other material.

The centrifuge method has the advantage of mathematical treatment. Its limitations are that it cannot differentiate between localized regions of a cell; that is to say, it gives a value of the cell as a whole, and no cell is of uniform consistency throughout. There is also the possibility that a force three hundred to five thousand times that of gravity may cause injury, with a resulting change in consistency. Weber believes that centrifuging produces no injury to protoplasm, yet Němec has demonstrated that it does considerable damage to cells that are dividing. Perhaps both workers are correct, and the disagreement in their results is due to the kind of cell and the physiological state of the protoplasm. The cells that Weber used were the vegetative ones of the alga *Spirogyra*, which are probably less sensitive than the eggs used by Němec, because the latter were in the process of division, a time when an ovum is certainly very sensitive to mechanical disturbances. D. Kostoff has induced various abnormalities in mitosis and even breakage of the chromosomes by centrifuging.

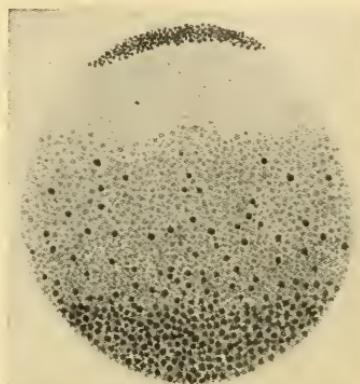


FIG. 108.—A centrifuged *Arbacia* egg showing stratification of the protoplasmic granules; the pale, centrally located bodies are yolk-spheres; the black bodies massed at the one (upper) pole are oil globules; the dark bodies at the opposite (lower) polar region are mitochondria. (From E. B. Wilson.)

It has been shown that bacteria may be centrifuged at high speeds and yet live. On the other hand, A. R. Moore finds that slime mold protoplasm may be severely injured and growth retarded by centrifuging. The problem of possible injury caused by a method of investigation, and the resulting pathological changes that may occur is one to which most methods of determining protoplasmic consistency are subject.

A further limitation of the centrifuge method (but by no means peculiar to it) is the impossibility of ascertaining with any exactness the values needed for computing viscosity—in this case, from Stokes' formula—such as the density of the dispersion medium (the protoplasm) and the density and radius of the suspended particles, which are mere specks under the highest powers of the microscope. Any estimation of these values is likely to involve a large error. There is also the question whether or not Stokes' law or any of the single constant laws of true viscous flow (of Newton, Maxwell, *et al.*) based on pure fluids hold in the case of protoplasm any more than they do for gelatin and other substances which show anomalous behavior (page 227). This question is probably best answered, as it is in the case of the applicability of the gas laws to solutions, by regarding the laws as fairly accurately applicable at low dilution, *i.e.*, low viscosity, but not at high concentration, *i.e.*, high protoplasmic consistency. When the centrifuge method is applied to thin protoplasm, it probably gives fairly accurate values, which have the great advantage of being capable of experimental reproduction. L. V. Heilbrunn has applied the centrifuge method to studies of protoplasmic consistency.

The Brownian-movement Method.—Brownian movement is the zigzag motion exhibited by all colloidal particles when suspended in a medium of not too high consistency (less than that of glycerin). The smaller the particle and the thinner the medium the greater the motion; therefore, the rate of the Brownian movement of included particles is an indication of the degree of viscosity of the medium. But this is accurate only for pure liquids and true solutions. In polyphasic systems such as protoplasm, the movement of particles in one of the phases does not necessarily throw light on the state of the mass as a whole; thus, Mast and others have shown that there may be very active movement of particles in highly viscous proto-

plasm because the particles are suspended in minute vacuoles the contents of which are of low consistency, the vacuoles themselves being embedded in a firm jelly, *viz.*, the living protoplasm. While these facts make it necessary to use Brownian movement as an indicator of protoplasmic viscosity with caution, they do not altogether nullify the observations that have been made by this method, because not all cell particles are in vacuoles; many are suspended in the protoplasm.

In a quiet Amoeba, the number of particles in motion, which are suspended directly in the protoplasmic matrix (*i.e.*, not in vacuoles), are few, and the amplitude of their motion is small. The viscosity of the protoplasm is, therefore, high. The consistency of glycerin is just high enough to prevent the motion of suspended carmine granules. The latter approximate the dimensions of protoplasmic particles; therefore, the viscosity of the quiescent protoplasm of Amoeba is somewhat less than that of glycerin, *i.e.*, between seven and eight hundred times that of water. In an active Amoeba, all particles, except the largest droplets, are in motion. The viscosity of the protoplasm is then low.

Bayliss used Brownian movement as an indicator of the sol and gel state of Amoeba when the latter is subjected to an electric shock. The electric shock causes the protoplasm to gelate, and all Brownian movement of particles ceases. When Amoeba recovers, the protoplasm solates, and the particles again take up their active motion.

W. H. Lewis finds that Brownian movement may be present or absent in tissue-culture cells, indicating considerable variation in the consistency of the cytoplasm, ranging from "fluid" to "semisolid." Particles in the cells of smooth and skeletal muscle exhibit little movement and in heart muscle none at all.

Bass-Becking has made a mathematical analysis of the Brownian movement of particles in Spirogyra and finds that the consistency of the protoplasm of this alga varies from that of water to several hundred times that of water, all within a very small region. He concludes that protoplasm has no single viscosity value but a large number of variable viscosities.

The Electromagnetic Method.—Several workers have attempted to determine the consistency of protoplasm by the electromagnetic attraction of a metal particle embedded in it.

The magnetic force (as measured in terms of amperes of current applied to the electromagnet) necessary to set the metal particle in motion and the rate of movement of the particle while it is being pulled through the protoplasm by the magnet are measures of the viscosity of protoplasm. The method substitutes an electromagnetic force for that of gravity in the fall method and that of a centrifugal force in the centrifuge method. The metal (iron or nickel) particles, if very small (7μ), must be inserted with the aid of a micromanipulator (Fig. 37). The results are only fairly accurate, for the experimental error is great. The method is of interest chiefly because of the novelty involved in getting a minute metal particle freely suspended in living protoplasm and then attracting it with an electromagnet.

The Microdissection Method.—The determination of the consistency of protoplasm by the manipulation of glass dissecting needles is comparable to judging the consistency of a liquid by watching some one stir it with a stick. In a sense, no method could be more crude, yet it is possible thus readily to distinguish differences in consistency between, for example, water, a thin oil, bread dough, and butter. It requires but little practice, by actual experimental test with solutions of differing concentrations of gelatin, to distinguish in the same way but with the use of a very fine glass needle, 10 viscosity values, from that of water to that of a firm jelly. Such a scale of values, determined by microdissection, has been established for protoplasm. The criteria by which one judges are the distance from a moving needle at which particles are disturbed and the length of time that deformations maintain their shape. For example, a furrow made across an Amoeba may close slowly, indicating high viscosity; or quickly, indicating low viscosity.

The simpler and more direct way of doing a thing, even though crude, may be the more exact one in the end. The application of physical laws and mathematical formulas leads to greater accuracy *provided* the laws and formulas hold for the intricate system being investigated, as they do for the simpler systems on which they were founded. The great difficulty with protoplasm, which so often upsets measurements of its physical properties, is its lack of homogeneity. Most methods for determining viscosity are based on the assumption that the material is homogeneous, which is never true of protoplasm. In this

respect, the microdissection method has some advantages, as it permits the determination of the viscosity of the most minute regions in a protoplasmic mass as well as of larger parts, or "organs," of the cell, the nucleus, the chromosomes, and the protoplasmic membrane.

The question of change in viscosity due to injury is again applicable, as it is in every method involving the application of instruments or the adding of reagents to protoplasm; but the micrurgist, with his material constantly under observation, soon learns to recognize the first sign of abnormality, such as a sudden change in viscosity, in color, or in granulation. Protoplasm sometimes disintegrates instantly at the slightest touch, but it also often tolerates a great deal of handling without evident ill effect and usually gives quick notice when there is change to the abnormal.

VALUES

Anyone reviewing the work done on the viscosity of protoplasm might become discouraged at the results, so great is the range in values, but this is just as it should be. Protoplasm may be of any viscosity above a minimum of ten to twenty times that of water, to the practically infinite value of a firm jelly.

Values between 800 (that of glycerin) and 8,000 (that of a thick sugar syrup) represent the more usual ones for fluid protoplasm, though a lower value of 10 or 20 may occur. Bread dough well illustrates the higher viscous state of protoplasm. The maximum values of protoplasmic consistency are reached when gelation (or coagulation) and subsequent dehydration take place. This is true of all protoplasm that is to undergo rest for a long time, as in dormant seeds. An extreme upper value is that of the resting plasmodium of a myxomycete (slime mold). During the winter, the plasmodium of a myxomycete becomes as hard and as brittle as a thin sheet of dry gelatin. It is difficult to realize that this so-called *sclerotium* of a myxomycete is a piece of living protoplasm, yet bits of it quickly start active growth when put in a moist and warm environment with food, even after being kept in the laboratory for some ten years. The viscosity of such a brittle sheet of protoplasm is infinite. While the dormant protoplasm of a slime mold represents an extreme case of protoplasmic consistency, yet an active plasmo-

dium may be of fairly high consistency. This is particularly noticeable when large masses of it hang freely suspended from the sides of a culture dish (*A*, Fig. 109) or form substantial columns between roof and floor of the culture dish (*B*, Fig. 109). Within such a rope of protoplasm, the inner mass is actively streaming.

There has been some unnecessary confusion in regard to viscosity values of protoplasm. One group of workers insists on low values, while the other lays emphasis on high. Both are right in part, as protoplasm runs the entire range in viscosity

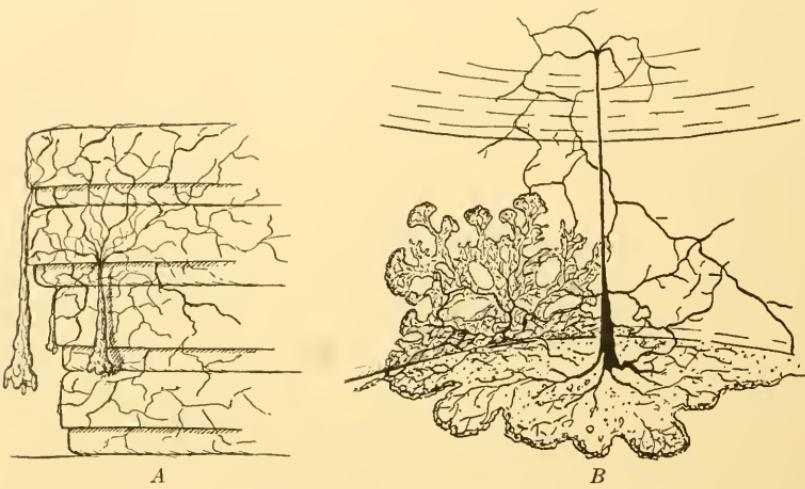


FIG. 109.—Ropes of protoplasm; *A*, hanging freely; *B*, forming a column.

(with the exception of the very lowest values), but there is a preponderance of higher values. Scarth compares the matrix of plant cytoplasm to a soft gelatin jelly and adds that even in its streaming condition protoplasm is viscid and highly extensile. This it would have to be in order to maintain strands freely suspended which yet show streaming within. Mudd uses the irregular form which white blood cells (leucocytes) maintain, even when subjected to tension, as an indication of the very high viscosity of the protoplasm. Runnström finds one (unfertilized) echinoderm egg (*Echinocardium*) to have a high viscosity value, while another (*Arbacia*) has a much lower value.

It is impossible to draw general conclusions as to the viscosity of protoplasm from one kind of material. The fact that protoplasm flows suggests low consistency; but strand formation, tensile strength, irregular form, imbibition, elasticity, and adsorp-

tion all indicate either high consistency or basic structural properties such as are characteristic of gels. It may be that those structural features, which are typical of jellies and responsible for their elasticity, sometimes lead to the false interpretation that the protoplasm is of high viscosity. For example, a moving particle, either a metallic one magnetically attracted or another centrifugally thrown through a jelly (gelatin or protoplasm) will be hindered in its movement by both viscosity and structural features. The first may be low, and the latter wholly responsible for the slow movement; thus will a high viscosity value be obtained when actually only structural resistance has been encountered.

The opposing views on the viscosity of protoplasm may be explained in a similar way. One method (microdissection) reveals the viscosity of the mass as a whole, while another (Brownian movement or centrifuging) indicates the viscosity of only one phase of the system—the aqueous dispersion medium. One may think of a sponge; as a whole it is moderately firm, yet particles suspended in the water which permeates it would move through it with the same ease as through water, for that is what they are in. A situation comparable to this exists in certain gels which are quite rigid to low stresses but offer no resistance to the diffusion of substances through them. Somewhat similar is the behavior of starch. If one's finger is moved slowly through a mixture of starch and water, the impression of a very thin liquid is gained; but if the finger is pulled up suddenly, it sticks as in cement. One is dealing here with a "brittle liquid" which can be powdered by rubbing between the fingers but will flow again as soon as left to stand a while. Slow movement of the finger leads one to believe that the starch mixture is of low viscosity, while rapid movement indicates high viscosity. The same behavior is to be observed in wet quicksand. The foregoing phenomenon is a thixotropic one (page 150), such as is characteristic of a number of gels. If zinc oxide is mixed with alcohol (in about equal parts) and transferred to a closed container, it appears to be rigid, and the vessel may be turned upside down without there being any tendency for the mixture to flow. But if the vessel is shaken vigorously, the mixture splashes about like water. Protoplasm also shows thixotropic reactions, and its general behavior is that of gels of this character.

Changes in Viscosity.—Protoplasm passes through a wide range of viscosity values in its normal life. These changes are coincident with changes in physical and physiological activity. When protoplasm is in an active state of streaming, of growing, or of metabolism, its viscosity is relatively low. Resting protoplasm is of high viscosity. The protoplasm of the growing mother cell of the egg of the seaweed *Fucus* is of low consistency. As development progresses, the consistency increases until at maturity, when the eggs are in a state of rest awaiting fertilization, their inner protoplasm is moderately viscous, and the cortical region a soft gel. As the plasmodium of a slime mold prepares to fruit, its consistency gradually increases until a firm jelly exists throughout.

It can be stated as a general rule that a state of high metabolic or physical activity means low protoplasmic consistency, and low metabolic or physical activity means high consistency. This statement should not be taken to mean that viscosity *determines* metabolic rate or any other activity, such as protoplasmic streaming, but simply that low viscosity is coincident with an active state. Protoplasmic streaming is certainly facilitated by a decrease in viscosity, and it is usually accompanied by such a change, but the decrease in viscosity is not the cause of the active streaming.

Changes in protoplasmic consistency occur during cell division. The pioneer worker in this field is Němec. He found an increase in consistency during the early stages of mitosis. At the completion of division, the viscosity of the protoplasm reverts to the original fluid state. Němec was able to discern regional differences in consistency at mid-mitosis. Increase in consistency is greatest at the periphery of the cell—so great in some cases that the outer cortical zone becomes a firm jelly enclosing an inner, more fluid region.

Following Němec, a number of workers studied viscosity changes during the mitosis of echinoderm eggs. Considerable confusion resulted until Fry and Parks carefully repeated the work, using the centrifuge method, and found that during mitosis of *Arbacia* and other eggs the viscosity is low during the early stages of mitosis, rises during metaphase (mid-mitosis), and reaches its maximum value during anaphase (late mitosis). At cleavage, it drops to the original value. The discrepancy in

results between workers may have been due to technique or to difficulty in distinguishing stages in mitosis in living eggs.

Changes in protoplasmic consistency can be induced by external factors such as temperature and salts. Those observed are the result of laboratory experiments, but many are probably duplicated in nature. The viscosity of gelatin falls with rise in temperature. Lecomte duNoüy found the same to be true for blood serum the viscosity of which falls from a value of 490 at 20°C. to 240 at 56°C. F. Weber finds that the viscosity of plant protoplasm decreases with rise in temperature.

The effect of salts on the consistency of protoplasm has been extensively studied, but there is little agreement in the experimental results. It does appear, however, that sodium ordinarily lowers viscosity and that calcium raises it. Other protoplasmic changes produced by these elements support this deduction, *viz.*, that sodium disperses, and calcium aggregates; thus, sodium increases the permeability of the cell, and calcium (usually) decreases it; calcium is necessary for membrane formation; *i.e.*, it coagulates or aggregates the surface protoplasm, while sodium has no influence.

As in the case of most experimental results obtained on the effects of salts on protoplasm, the statement that calcium tends to gelate (thicken) and sodium to solate (thin) is met with denial; however, the majority opinion holds that calcium increases protoplasmic viscosity while sodium either lowers it or has no appreciable influence. But no general rule can be stated, for there is no definite physicochemical entity that we can call protoplasm. Not only does the protoplasm of species differ but that of tissues and of adjoining cells as well. While so complex and varied a system as protoplasm is likely to show diverse viscosity changes brought on by the same salt, yet such may occur in much simpler (nonliving) systems and in so complicated a way as to give irregular curves; the same salt may increase and decrease viscosity.

Acids have an aggregating effect on protoplasm. Living nuclei may be coagulated by acid, as indicated by a granular appearance, and yet remain alive. The coagulation may be reversed by bringing the cell back to a neutral condition; normal growth then continues. Heretofore, coagulation has been regarded as

incompatible with life, but this appears to be true only when it is irreversible (if we may speak of reversible coagulation).

The effect of anesthetics on protoplasmic consistency has been extensively investigated. Lloyd finds that the application of low nonlethal concentrations of narcotics (ethyl alcohol, ether, chloroform) is accompanied by reduced viscosity (of *Spirogyra*), while higher concentrations increase the viscosity presumably by dehydration. Other workers find a pronounced increase in protoplasmic viscosity when narcotics are added.

Electricity produces an increase in protoplasmic consistency; the change, if not carried far, is reversible. Kühne, as early as 1864, used electrical stimulation as a procedure for the study of reversible viscosity changes in protoplasm. The interesting experiment of Bayliss on the gelating effect of a mild electric shock on Amoeba has been referred to (page 215).

Mechanical irritation may cause a change in consistency; it may bring about a complete collapse in the intricate structure of a dividing cell. An egg in mid-mitosis may, on puncture with a needle, completely collapse, leaving no vestige of the former asters and spindle. Such behavior is closely analogous to the thixotropic collapse of an iron oxide gel (page 150).

Death brings on marked changes in the viscosity of protoplasm. The usual change which accompanies death is coagulation, though often replaced or followed by complete disintegration.

Application to Physiology.—The importance of studies on the viscosity of protoplasm is indicated by the remarkably wide application of them to physiology. There is a correlation between the viscosity and the permeability of the plasma membrane. The formation, control, and repair of the protoplasmic membrane—indeed, all surface phenomena in the cell, such as the distribution of protein and fat substances—are to a great extent controlled by the viscosity of protoplasm. Amoeboid movement has long been regarded as related to, if not determined by, changes in viscosity. Viscosity changes have an important bearing on the dynamics of mitosis.

The mechanism of the operation of contractile vacuoles has to do with variations in consistency, as Taylor and Lloyd have shown. Gasser and Hill have discussed the role of viscosity in the dynamics of muscular contraction. Probably all forms of tissue contractility are associated with viscosity changes.

The knowledge that low concentrations of ether, chloroform, etc., decrease, while higher concentrations increase protoplasmic consistency, cannot but add to our understanding of anesthesia. It is of interest in this connection to recall that Claude Bernard regarded anesthesia as a reversible coagulation of the protoplasm of the sensory nerves (page 497). Růžička advanced the hypothesis (page 208) that aging is a matter of dehydration of protoplasm and, therefore, of decreased colloidality and increased viscosity. (In all such hypotheses, one must be careful to distinguish between cause and effect.)

H. J. Jordan has studied the bearing of viscosity changes on muscular action and finds that with stimulation the colloidal state of muscle (of the snail) is fully changed through the transformation of a fluid medium into a firm body which results in shortening (usually) and the production of tension. Jordan adds that the physical changes in muscle are dependent upon structural, elastic, and other like properties of protoplasm. Probably the viscosity changes in muscle are more correctly characterized as thixotropic (page 150).

The viscosity, specific gravity, osmotic pressure, and swelling of blood have been used by Ludlum and Nugent as indicative of a pathological condition. Patients suffering from asthenia (debility) show low values in the viscosity, etc., of their blood serum, while those suffering from delirium or toxemia show high values in viscosity.

One reiteration and one caution should conclude the experimental portion of this chapter. The reiteration is the statement that protoplasmic consistency is constantly changing. Any physical change (*e.g.*, streaming, coagulation) or any chemical change (*e.g.*, rate of oxidation, digestion, synthesis) is almost certain to result in a change in viscosity. Consequently—and here is the caution too often neglected—it is erroneous to select the only visible change, *viz.*, one in viscosity, as the characteristic effect of an external influence when viscosity may be the secondary result of any one of a number of primary changes.

THEORY

Viscosity vs. Plasticity.—Lucretius, two thousand years ago, knew that the rheologist had some knotty problems to solve. In his "Of the Nature of Things," he writes:

We see how quickly through the colander
The wines will flow; on the other hand,
The sluggish olive-oil delays: no doubt,
Because 'tis wrought of elements more large,
Or else more crook'd and intertwined.

How near right Lucretius may have been is shown by the recent work of Staudinger, which indicates that in a solution containing very long, threadlike molecules, normal movement is impeded, and high viscosity results. On this basis, he calculated the molecular weight of substances (rubber), presuming a very simple and direct relation to exist between viscosity and molecular size. That such a relation may exist is obvious, but high viscosity is not necessarily proof of large and linear molecules. Solutions of small molecules are often extremely viscous. The high viscosity and especially the irregular behavior of colloidal substances may be due to the aggregation of their molecules—in other words, to colloidal structural properties. Bingham interprets the high viscosity of substances with low molecular weights as due to "association" caused by "associating groups" such as NH_2' , OH' , and COOH' . Because of association, diethylene glycol, $\text{C}_2\text{H}_4(\text{CH})_2$, is very viscous, and urea, CONH_2 , is a solid, as is also oxalic acid, $(\text{COOH})_2$, with an extremely high melting point. Propionic acid, $\text{CH}_3\cdot\text{CH}_2\cdot\text{COOH}$, is a fairly mobile liquid, but hydrarylic acid, $\text{CH}_2\text{OH}\cdot\text{CH}_2\text{COOH}$, is a thick, syrupy liquid. This leads us to the interesting anomalous behavior of some solutions.

In 1685, Isaac Newton announced, in his "Principia," the law of fluid flow. He expressed it mathematically thus: $v = \phi Fr$, which means that the velocity, or rate of flow, v is proportional to the fluidity ϕ (the reciprocal of the viscosity) and the shearing stress F on either of two planes separated from each other by the distance r . Newton had confidence in his theory and never bothered to test it out experimentally, nor did anyone else for a century and a half. It remained for Jean Poiseuille, French professor of medical physics, to furnish proof of Newton's law. He found that the volume of flow of a liquid v is proportional to the time t , the pressure p , the fourth power of the radius of the capillary r , and a constant k , and inversely proportional to the length of the tube l . This relationship he expressed in the following formula which bears his name:

$$v = \frac{tpr^4}{l} k$$

The value of $k(\pi/8)$ is inserted in the more usual form of Poiseuille's formula:

$$v = \frac{\pi pr^4 t}{8l\eta}$$

Pure liquids and true solutions all obey Poiseuille's law and are said to be Newtonian, that is to say, to exhibit true viscous flow. But many solutions do not obey Poiseuille's formula; they are non-Newtonian and exhibit anomalous flow. They possess not one viscosity value but an infinite number. Why this is true is not known. The fact and its causes have been the center of a lively and informative discussion which led to the founding of the Society of Rheology through the activity of the American chemist Eugene Bingham. We cannot hope here to consider the technical and mathematical features of this difficult problem, but we can learn something of the nature of non-Newtonian substances and consider a possible cause of their anomalous behavior.

Practically all of the lyophilic colloids—the organic gel-forming elastic systems (*e.g.*, proteins)—exhibit variable viscosity; they deviate from Poiseuille's formula. If a pure liquid such as water or glycerin is run through a capillary viscometer at different pressures, the calculated viscosity value remains the same. The rate of flow v/t will change with pressure, but the viscosity η remains constant. All pure liquids have a definite viscosity value which is characteristic of them. But if a solution of gelatin, albumin, or soap is run through the capillary at different pressures, each experiment yields a different viscosity value until a certain maximum pressure is reached. Which of these variable viscosities is the correct one? The only one that has something in its favor is that obtained at high pressure, as it remains fairly constant with increased pressure.

The difference in behavior between a pure liquid or true solution exhibiting true viscous flow and a colloidal or other non-Newtonian fluid exhibiting anomalous flow is seen in a graph. The line OA (Fig. 110) is the graph of a Newtonian liquid where rate of flow is plotted as ordinates, and the pressure or shearing

stress required to produce it, as abscissas; the line OA is straight and passes through the origin, which means that the ratio of flow to force is a constant. The graphs OB and OC are those of non-Newtonian liquids; they are either curved or do not pass through the origin; the ratio of flow to force is a variable. Fluids exhibiting anomalous flow behave as if they contained aggregates which under stress are sheared apart, thus increasing the fluidity. Forces that hold molecules together in aggregates may also cause the material to show elasticity. For this reason, Freundlich and Reiner both assumed that elasticity is responsible for the deviation of flow from Poiseuille's law. Such a conclusion was

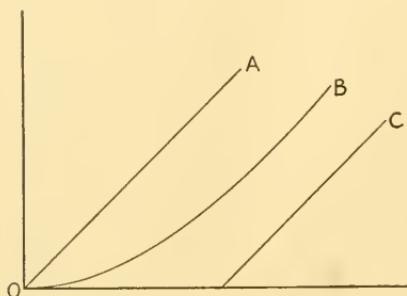


FIG. 110.—Graphs of true (OA) and anomalous (OB and OC) viscous flow (ordinates are rate of flow; abscissas are shearing stress).

shown to be justified from the behavior of two soap solutions the viscosity of which was measured in a capillary viscometer. One was found to obey Poiseuille's formula, while the other deviated from it; the former should not show elastic qualities, while the latter should. This proved to be true. Furthermore, the elastic soap possessed structural characteristics which permitted it to

hold a small metal particle in suspension though it was but twice as viscous as water, while the soap solution which showed true viscous flow would not support a similar particle even though four hundred times as viscous as water. The structural features that gave to the non-Newtonian soap its elasticity and rigidity were probably responsible for its non-Newtonian behavior. All typical lyophilic colloids which form jellies exhibit anomalous flow, and all are elastic. The American school of rheologists are inclined to dissent from this view, for they regard viscosity and elasticity as independent variables. Evidence in their support is the fact that certain other suspensions, such as pigments and clays, also exhibit anomalous flow yet are presumably inelastic. On the other hand, oils such as linseed, which when pure show true viscous flow, on oxidation set to an elastic jelly. Perhaps it must be granted that not all cases of anomalous flow are due to those structural properties that are responsible for elasticity,

yet not until there has been found an elastic colloidal system which exhibits true viscosity can elastic structure be disregarded as a possible factor in anomalous flow.

In an attempt to straighten out these difficulties, Bingham introduced a new interpretation of the word "plastic"—new because he applied it to liquids as well as solids. To be plastic in the Bingham sense, the ratio of flow to force in a liquid must be variable. Consequently, materials that give curved flow-pressure graphs are said to be plastic.

One of the characteristics of plastic, or non-Newtonian, liquids is the possession of a *yield value*. They require an initial force to start flow. Yield value is the minimum shearing stress in dynes per square centimeter required to produce continuous deformation. Sheppard, while accepting in the main the plasticity idea, holds to the opinion that non-Newtonian behavior is a criterion of molecular aggregation, or of structural features, such as account for elastic properties. This suggests that we may be dealing with two types of viscosity—true (inner friction) and structural.

These questions of theory, which are rather out of the province of the biologist and must be left to the rheologist to solve, need not seriously bother the worker on protoplasm, because the most that he can hope for is a rough approximation of the *consistency* (a safe term under all conditions) of protoplasm. However, the biologist must realize that these questions exist and that the material with which he works is not a pure liquid or true solution but exhibits anomalous flow and is therefore not obedient to the laws of Newton, Poiseuille, and Stokes.

Some workers will dissent from this last statement, which to others is as true as any that can be made of protoplasm. We read that protoplasm is a pure solution. The declaration is made by those who view it as a system of no great complexity. If this well-nigh inconceivable concept is for the moment accepted, we must at least give to protoplasm the same degree of anomalous behavior as that possessed by a protein or a soap solution, for the dry weight of protoplasm is half protein, with a fraction of soap, and neither exhibits true viscous flow.

Studies of flow under varying stresses show that colloidal solutions of the lyophilic type are mostly anomalous in character. The viscosity is to be regarded as "apparent" and should be

determined under several shearing stresses. It has been suggested that the type of plastic flow of most interest to the biologist may give an apparent fluidity φa , which will be a function of the shearing stress, as follows:

$$\varphi a = \varphi_0 + aF$$

where φa is the apparent viscosity at zero stress, and a is a constant.

The viscosity and in particular the anomalous behavior of protoplasm are part of the bigger problem of the structural organization of protoplasm.

CHAPTER XIV

ELASTICITY

Protoplasm is elastic. This property in itself does not appear to have so important a bearing on the physiological behavior of protoplasm as does viscosity. It appears rather to be the outcome of other more significant qualities. The chief interest in the elastic property of protoplasm lies in its bearing on the fundamental question of protoplasmic structure; elasticity is the best indication that we have of the structure of living matter.

Elasticity, in general, implies springiness and may be defined as that property of substances which causes them to assume their original shape after being deformed.

If a steel anvil is struck with a hammer, the hammer rebounds, and we say that the steel is elastic. An enclosed gas or liquid when compressed assumes or tends to assume its original volume when the pressure is released. The gas or liquid is elastic. When rubber is stretched and of itself returns, it does so because it is elastic. All three of these examples represent different forms of elasticity in the technical or popular sense. Gases and liquids possess an elasticity of volume; with this we are not concerned here. If elasticity is defined and measured in terms of the force necessary to produce maximum deformation from which a body will recover when the force is removed, then the greater the force and the more perfect the recovery the greater is the elasticity; in this sense, the steel anvil is more elastic than the rubber band. Rubber is of high *extensibility*; this is the proper term when *stretch* is referred to. There is, however, a type of stretch that does not involve recovery and, therefore, elasticity; this is ductility. Lead is highly ductile and therefore extensible, but it shows no (or very little) recovery and is consequently not elastic.

There are a number of other properties closely associated with elasticity which are equally characteristic of protoplasm but are

not to be confused with it, even though they may rest upon similar structural features. Such properties are contractility, rigidity, cohesion, adhesion, tensile strength, toughness, stickiness (tackiness), and glutinosity. It should be pointed out that these properties, including elasticity, have no necessary relationship to viscosity. Elasticity may interfere with the accurate measurement of viscosity, but it does not rest upon the same mechanism. A change in one may, but need not, involve a corresponding change in the other. Glycerin is highly viscous yet possesses no elastic qualities (other than bulk elasticity). A very thin soap solution, with a viscosity value barely more than that of water, may be quite elastic (*i.e.*, extensile). Such a solution also possesses rigidity—an unusual property to ascribe to a liquid—but it, like elasticity, is a property of (certain) liquids.

Hatschek has called attention to the fact that if a soap solution is quite thin, its elastic qualities (if it possesses any) may be demonstrated by putting it into a flask which is then given a quick turn, or swish; the solution will swirl around, come to a rather sudden stop, and return a short distance. Had the liquid been inelastic (in the sense of stretch and recovery), as are water and glycerin, it would have come to a standstill slowly, without showing any tendency to return. The elasticity of thin solutions and at the same time their rigidity may be demonstrated in yet another way.

If a liquid possesses structural features that enable it to keep a metal particle within it in suspension, this quality may be used as a means of measuring the stretching capacity of the liquid. A microscopic (15μ) metal (nickel) particle is placed in the liquid, *e.g.*, a soap solution, with the aid of a micromanipulator. The particle, as it remains in suspension, is then attracted by an electromagnet (Fig. 35). If the particle, on release of the magnetic field after having been drawn through the solution, shows any return, whether wholly or in part, the solution is elastic. A particle suspended in glycerin stops precisely where it is when the magnetic field is eliminated, because glycerin is inelastic; but in certain soap and other elastic solutions, the particle returns.

The elastic and rigid qualities of thin solutions are well illustrated by the behavior of soap solutions. A 0.1 per cent solution

of sodium stearate (of the right quality) with a viscosity value but twice that of water will hold a minute metal particle in suspension and exhibit a high stretching capacity, while a 40 per cent sodium oleate solution, four hundred times as viscous as water but wholly inelastic, will not (permanently) hold the same particle in suspension. Two samples of sodium stearate proved extraordinary in that, though presumably alike chemically, they exhibited quite different physical properties. (They came from different manufacturers.) Freundlich and Schalek had found that one of them deviated from Poiseuille's law of true viscous flow, while the other obeyed it. The first proved to be elastic, and the latter not. The elastic soap held a metal particle in suspension even though the viscosity of the solution was but twice that of water, while the inelastic soap required a viscosity value of 31 times water to support the same particle barely long enough to permit a hurried measurement to be made. Their microscopic structural features also differed (page 226). These facts teach us that deviation from Poiseuille's law (*i.e.*, anomalous flow), elasticity, and rigidity are usually concomitant and that when one is absent, the other is also likely to be absent. How general this rule is cannot be said, but it seems always to apply to such gel-forming substances as soaps and the hydrophylic colloids in general, including protoplasm.

Elasticity, rigidity, and the related properties of contractility, tensile strength, etc., are characteristic of protoplasm. Their presence there can be experimentally proved. They indicate that anomalous flow is also a characteristic of protoplasm.

Protoplasm was early known to be elastic and sticky. The first attempt at an accurate determination of one of its mechanical properties was made by Pfeffer, who ascertained the tensile strength of a strand of myxomycete protoplasm by attaching weights to it and found that a strand measuring 0.3 mm. in diameter would support 3.5 mg., which is 50 mg. per square millimeter.

The elastic qualities of protoplasm can be easily demonstrated and measured with fair accuracy. When protoplasm is thin and the extensibility correspondingly low, the presence of elasticity cannot be readily shown, but there is no reason to assume that it has therefore disappeared. The chemical constitution of the dry weight of protoplasm is half protein; this indicates that

elastic properties must be present, as proteins are, without a known exception, elastic.

Attention is called by Searth to the remarkable elastic and rigid properties of protoplasm. He shows that strands of streaming protoplasm may be so rigid that when slowly stretched (by swelling in water) they suddenly snap across and then crumple up on the recoil like a solid thread and not like a fluid. Yet almost immediately they show fluid properties again in that the protoplasm begins to stream.

The simplest and most convincing way to demonstrate the elasticity of protoplasm is to stretch it between microneedles.

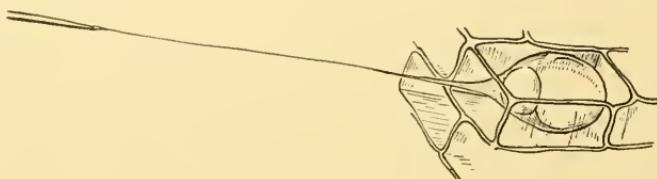


FIG. 111.—The stretching of protoplasm with the aid of a microneedle.

One may or may not, in such an experiment, be handling living protoplasm. Usually, however, one can tell fairly well in what condition the protoplasm is. Death is ordinarily accompanied by coagulation, and a coagulum is poorly extensile. Living protoplasm may be stretched into very fine threads. The epidermal cells of onion leaves are good material for such a study. The cells are plasmolyzed (the protoplasm shrunken away from the cell wall), and the tissue cut across, thus opening some of the cells without touching the protoplasm within (page 61, Fig. 8). The cells may then be entered by a needle, and the naked protoplasm touched. If the protoplasm is sufficiently glutinous (sticky), as it usually is, it will adhere to the needle and may then be drawn out to great lengths (Fig. 111). When the protoplasmic thread snaps, its elastic limit has been passed. A stretched protoplasmic thread may become so fine as to be invisible under the highest power of the microscope. Evidence of its persistence is given by the presence of globules along its length, which continue to move apart from one another and away from the cell as the needle is withdrawn. If stretching is continued, the thread eventually breaks and then contracts, moving so rapidly at first that it may double back on itself like

a taut rope suddenly severed. Finally, the stretched and now contracted protoplasmic mass is slowly reincorporated into the protoplast. This is a good indication that it is still living. Further evidence that stretching does not necessarily cause severe injury is to be had in active streaming. Protoplasmic streaming often remains undisturbed in a cell after parts of it are stretched

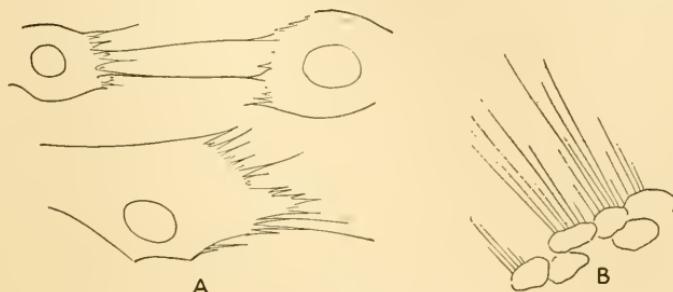


FIG. 112.—Protoplasmic strands formed by fibroblasts and other cells in tissue culture.

to twenty times the original length or more. The elastic limit of protoplasm may serve as a measure of the effects of salts and other chemicals (page 448).

That the elastic quality of protoplasm as demonstrated by stretching with microneedles is by no means an abnormal one is convincingly shown by the snapping and rapid contraction of cell processes (pseudopodia, protoplasmic threads, and the like) formed naturally by cells.

This is to be seen in tissue cultures in which fibroblasts form long protoplasmic protrusions which adhere to the glass as the cell moves forward, then stretch and snap (Fig. 112). Observations of leucocytes by S. Mudd show that the elasticity and tensile strength of cell processes may be very great (Fig. 113). In all such cases, the elastic quality is indubitably a normal property, and it is identical with that demonstrated by micromanipulative methods.

Entire cells, without cellulose walls, such as red blood corpuscles (Fig. 47), may be stretched between microneedles. Where an entire cell is stretched, it is the elasticity of the cell membrane that is determined; in fact, this is usually (but not always) the case; however, the plasma membrane is protoplasm.



FIG. 113.—A leucocyte "pursuing" an abnormal red blood cell until almost stretching itself in two. (*Sketch from a photograph by S. Mudd.*)

A number of interesting facts pertaining to problems other than those bearing on elasticity as such, *e.g.*, permeability (see Fig. 47), are revealed by stretching cells.

Nuclei may likewise be stretched. When isolated, a nucleus is always abnormal, if not dead. The nucleoplasm is then often very extensile, though it may tear like butter (Fig. 48). When the nuclear plasma is elastic, it is probably alive; and when inelastic, it is coagulated and dead.

The elasticity of protoplasm varies greatly. It may be artificially changed by the addition of salts (page 448). The protoplasm of an onion cell which has rested overnight in sodium nitrate has an elastic limit much lower than normal, while protoplasm treated with a calcium salt is considerably more elastic than that of the control cell. Magnesium has no effect. Acid lowers the elastic (stretching) limit. Sodium usually decreases viscosity and elasticity, while calcium increases both properties, but acid increases viscosity (coagulates) and decreases elasticity (extensibility).

The value of elasticity measurements of protoplasm lies in the fact that stretch is one of the few mechanical properties of protoplasm that can be determined with fair accuracy and thus serve as a reliable indicator of the effects of reagents and of other experimentally induced changes in protoplasm. Elasticity is also that property of protoplasm which gives the best indication that we have of the finer structure of living matter. As Scarth says, elasticity and the other jelly properties of protoplasm afford "the only available basis for conjecture as to its ultra-microscopic structure," and McBain is of the opinion that the presence of elasticity in a colloidal solution is a specific and positive test for the presence of ramifying aggregates. Continuity in the structure of protoplasm is an important physical concept about which we shall have more to say later; one evidence for it lies in elastic properties.

Most colloidal hypotheses of muscle action involve a consideration of elasticity. H. J. Jordan makes elastic and structural qualities the basis of his interpretation of the behavior of snail muscle. This muscle may be stretched a short distance and show perfect return (elasticity), but when elongated beyond a certain point, there is no contraction whatever; the elastic limit has been exceeded, causing a breakdown in structure.

Jordan compares the behavior to that of unvulcanized rubber, which may be stretched 100 per cent and yet show perfect return but if elongated beyond this elastic limit shows no elastic qualities. The structure responsible for the return has been destroyed. Beyond the elastic limit, there is a true viscous flow of the highly viscous fluid phase in which the micellar units are embedded. At the elastic limit, the grouping of the micelles, and therefore the structural continuity, of the elastic jelly is destroyed.

CHAPTER XV

THE STRUCTURE OF PROTOPLASM AND ORGANIC COLLOIDAL MATTER

The structure of protoplasm is one of the essentially physical qualities of living matter, comparable to elasticity, viscosity, and those other qualities that we have just been considering. It is, therefore, rightly grouped with these similar physical properties, yet for a complete understanding of it many facts to be considered later are necessary. We must decide between

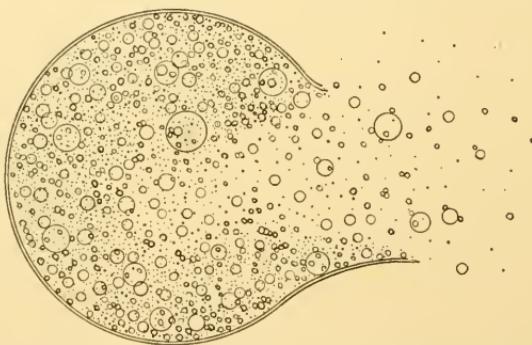


FIG. 114.—The coarse protoplasmic emulsion as seen in the egg of *Fucus*.

continuity in subject matter and the anticipation of much that is to come. We select the former and suggest that this chapter be reread after completing the book, not only for a better understanding of it but also because theories on the structure of protoplasm represent the culmination of all our knowledge of living matter.

THE VISIBLE STRUCTURE OF PROTOPLASM

Theories on the structure of protoplasm fall into two categories—those having to do with the microscopically visible structure and those dealing with the structure that lies beyond the visible. The first is based on direct observation; the second, on interpreta-

tion. Viewed through a microscope, protoplasm usually presents the picture of a fine dispersion of globules and particles of different sizes, freely suspended in a liquid medium (Figs. 114, 3). Many of the particles are mere specks; others are relatively large (maximum, 10μ) and are distinguishable as droplets. When the specks predominate, the protoplasm may, though possibly inexactly, be characterized as a suspension of granules. If, however, the specks prove to be globules, as are the larger particles, then the structure is that of an emulsion. These

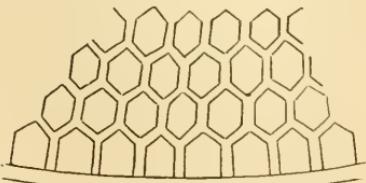


FIG. 115.—Alveolar protoplasm, sketched from a living Euplotes.

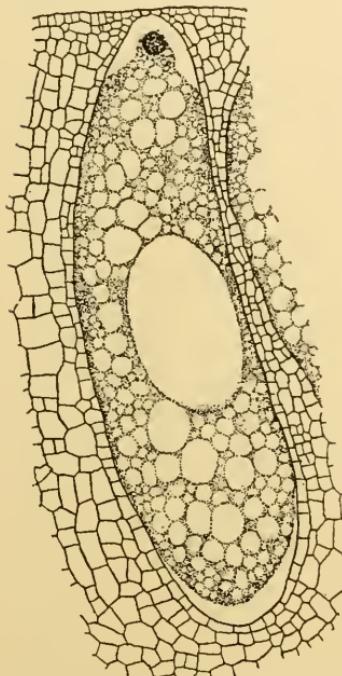


FIG. 116.—Vacuolate protoplasm of the egg cell of Ceratozamia. (After C. J. Chamberlain.)

possible configurations of the visible parts of living matter are the bases of three theories of protoplasmic structure, *viz.*, *granular*, *emulsion*, and *alveolar*. The propounder of the last mentioned theory introduced the word *alveolus* (a small *alveole*, or cavity) to indicate symmetrically arranged globules which resemble small vacuoles and assume, because of pressure, an angular form (Fig. 115). Neither *alveolus* nor *vacuole* is a well-chosen term, as there are no cavities or empty sacs in protoplasm.

Modifications of the alveolar or emulsion structure of protoplasm occur, though the differences are mostly superficial. When alveoli are not under pressure, and therefore less symmetrical (Fig. 3), they are referred to as *alveolar spheres*. When the "cavities" are of the nature of small sacs, the structure is termed *vacuolar* (Fig. 116). It is,

however, impossible to differentiate clearly among a *vacuole*, a *sac*, a *globule*, an *alveolus* or an *alveolar sphere*. All of these structures are but modifications of one and the same

thing, *viz.*, a droplet; they all become, therefore, emulsions in one form or another. Even the granular hypothesis now belongs under this heading, for Spek has seen the most minute of protoplasmic granules fuse and form larger liquid globules with discernible contours. (Some solid granules, such as crystals, occur in protoplasm.) We thus recognize one main type of visible structure in living protoplasm, *viz.*, an emulsion. The various distinctive names which have been given to this structure indicate, in part, the actual particular configuration assumed by the emulsion and, in part, the author's interpretation of it as it exists in the particular material he has investigated.

The Granular Hypothesis.—The observation of Spek just referred to indicates that protoplasmic "granules" are usually fluid droplets, as it has long been suspected that they might be, for *solid material is not compatible with life*; but granules they were in the minds of the older investigators. Protoplasmic granules were called *microsomes* by Hanstein (1882). Bütschli remarked that they thereby obtained the right of entry among the privileged and recognized units of protoplasmic structure, for "anything that is called by a Greek name at once seems to many people to be much better known, and as something which must be definitely reckoned with." What Bütschli says is quite true, but, after all, the granules must be reckoned with for reasons other than their Greek name. Altmann, who is usually regarded as the author of the granular hypothesis of protoplasmic structure, went so far as to suggest that the granules may be living bacteria. The cell thus became a colony of minute organisms. The idea that certain protoplasmic inclusions are bacteria appears again and again in biology. Mitochondria were once regarded as such and were therefore assumed to have a certain autonomy of their own apart from the cell. The rapid motion of rod-shaped mitochondria in a living tissue-culture cell (fibroblast) certainly suggests that these inclusions get about quite independently. However, that they are bacteria seems unlikely.

Of the physical and chemical constitution and physiological significance of protoplasmic granules little is known. Undue importance has been attached to many of them, and it is, after all, as Harper says, a motley collection which is brought together

under the head of granules, which include particles, vacuoles, plastids, secretory granules, mitochondria (spheres, rods, and threads), chromatin granules, globules of oil and yolk, crystals of salt and sugar, grains of starch, etc. Some of these are probably of vital importance; others serve as food; and still others may be waste matter. As a general theory of protoplasmic structure, the granular hypothesis is not a satisfactory one upon which to interpret cell behavior. The reasons for this will appear as other structural theories are discussed.

The Emulsion Hypothesis.—There is no doubt but that protoplasm, superficially viewed, is an emulsion. Doubt exists only as to the function and fundamental nature of this emulsion. The emulsion hypothesis got a firm hold in biology because protoplasm as seen through the microscope is quite evidently an emulsion, and because the colloidal structure of jellies was once thought to be a fine emulsion (the latter idea gave rise to the misleading word "emulsoid"). It was very natural, therefore, for biologists to assume that the ultimate and hidden structure of protoplasm is an emulsion, just like the coarser and visible one, only finer. Let us first recall what happened to the emulsion hypothesis of the structure of jellies. Donnan and Ellis found that fine and pure emulsions are "model suspension colloids" and not of the jelly type at all. Hatschek, in a search for a possible mechanism in emulsions which would explain such gel properties as elasticity, analyzed the situation mathematically and concluded that "the theory that gels consist of two liquids must be pronounced untenable."

A substantial support of the emulsion hypothesis of protoplasmic structure came from Clowes, who evolved an ingenious theory of protoplasmic permeability (page 287). He assumed that the outer layer of protoplasm is a fine (ultramicroscopic) emulsion near the reversal point. When the emulsion swings slightly to one side or the other, it becomes more or less permeable to water-soluble substances—more so when an oil-in-water emulsion and less so when of the water-in-oil type. The hypothesis nicely explains certain features of the permeability of protoplasm. Particularly convincing is Clowe's discovery that the proportion of sodium and calcium that keeps an emulsion at the reversal point is exactly that which exists in sea water, blood, and other physiological solutions. Such and other

evidence are sufficient to recommend the emulsion hypothesis of protoplasmic structure to many sound workers (page 288).

There can be no doubt but that the protoplasmic emulsion plays an important role. It presents a multitude of surfaces, and it is at surfaces that reactions take place. But there are a number of very characteristic properties of protoplasm which cannot be explained on the basis of emulsion structure. Protoplasm is elastic, and emulsions not (when pure). Protoplasm coagulates, and emulsions do not. When milk coagulates, a protein (caseinogen) coagulates and not the emulsion of butterfat. Emulsions exhibit phase reversal, and on this property does the emulsion theory of protoplasmic behavior rest, yet *there is no evidence whatever of phase reversal in protoplasm.*



FIG. 117.—Red blood cells of *A.* Triton, *B.* *Cryptobranchus*, with nuclei, showing alveolar structure. (*A.* from J. Comandon.)

It is very improbable indeed that the dispersion medium of protoplasm could become the dispersed phase (pages 288–289). Electrical conductivity measurements by Gelfan reveal that the conductivity of protoplasm is the same *at all viscosity values*. Blood also shows no change in conductivity in spite of a great increase in viscosity (as a result of coagulation). Arrhenius found this to be true of gelatin when it sets from a solution to a gel, and on this basis McBain denied the possibility of phase reversal when soap jellies are formed. The whole idea of phase reversal has been discarded as a property of gel-forming systems.

We may now consider some of the special forms that the visible protoplasmic emulsion assumes.

The Alveolar Hypothesis.—The German protozoologist Bütschli observed a very symmetrical honeycomb or checkerboard appearance in the protoplasm of certain organisms. He regarded this structure as fundamental. It seemed to be made up of tightly compressed globules which, owing to pressure, become angular, their geometrical shape being that of dodeca- or tetrakaidecahedrons. In optical cross section, the globules present a hexagonal outline. Bütschli called them *alveoli*. The structure is plainly visible in certain Protozoa, such as Euplotes

(Figs. 28, 115). It is also typical of some nuclei such as the macronucleus of *Euplotes* and the nuclei of amphibian (*Triton* and *Cryptobranchus*) red blood cells (Fig. 117). How alveolar protoplasm results from an emulsion under pressure is indicated in Fig. 118, where *A* schematically represents the usual protoplasmic emulsion; *B*, alveolar spheres; *C* or *D*, alveolar protoplasm; and *E*, a possible orientation. The alveoli become pentagonal and line up in very symmetrical order when adjoining

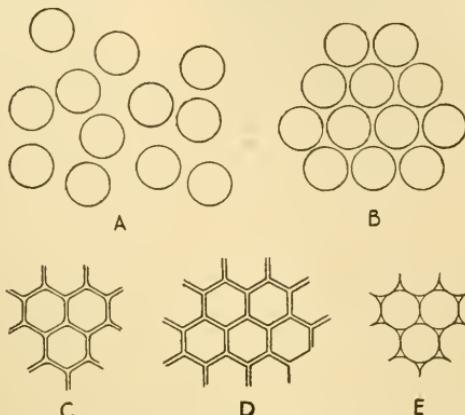


FIG. 118.—Five possible arrangements of globules in an emulsion.

an outer surface or a larger foreign particle in protoplasm (Fig. 115).

The criticism that has been directed against the alveolar hypothesis cannot be based on the contention that the structure is an artifact. This form of criticism arose from work by Hardy and Fischer, who showed that an alveolar structure can be produced in gelatin by treatment with certain reagents. It was, therefore, maintained that the structure is artificially produced in protoplasm. This is not wholly true. Bütschli's error did not lie in the observation of artifacts but merely in claiming a universal occurrence and fundamental value for the structure that he saw, in both living and nonliving material. An alveolar structure occurs in protoplasm but not in all protoplasm and not in gels.

The alveoli of protoplasm are probably minute vacuoles, for vacuoles are very abundant in protoplasm—more so than usually realized. Chamberlain has suggested that most emulsion, alveolar, and like structures of protoplasm are all modifications

of a vacuolar structure (Fig. 116). This may be true, but as vacuoles, like alveoli, are droplets of liquid, we can say that all forms of the microscopically visible structure of protoplasm are modifications of an emulsion.

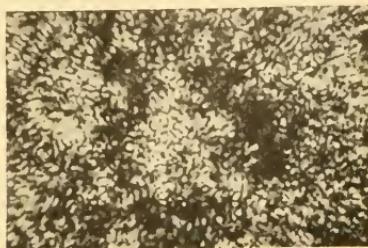


FIG. 119.—Quiescent protoplasm (slime mold) as seen through the Spierer lens.

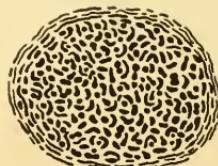


FIG. 120.—Sketch of a plant-cell nucleus as seen through the Spierer lens.

Protoplasm, as Bütschli contended, is usually if not always of a very fine emulsion structure, even though such a structure is not visible with ordinary optical methods. That this is true is indicated by the following observation. Protoplasm, which appears to be a homogeneous, hyaline substance, free of granules,

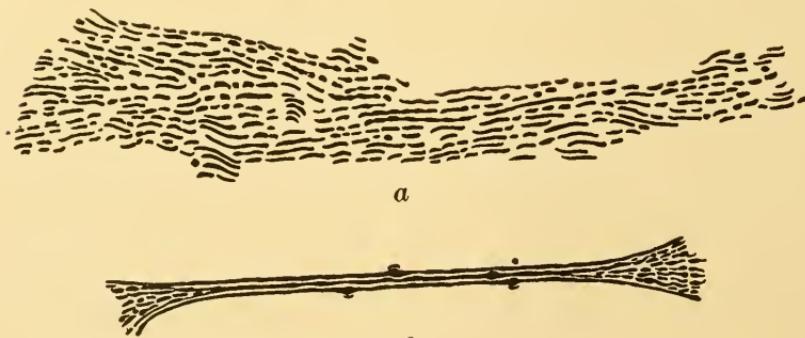


FIG. 121.—Representation of protoplasm as seen through the Spierer lens; (a) streaming; (b) as a thread.

may be revealed as a very fine emulsion when viewed with the Spierer lens (page 98). The one (dispersed) phase of the emulsion is brightly illuminated, while the other (dispersion medium) remains dark. When the protoplasm is quiet, the two phases present a mottled picture, a mosaic (Fig. 119). The plant-cell nucleus is of a similar appearance (Fig. 120); here the structure is often visible with ordinary (direct) illumination.

When the protoplasm is under tension, as when formed into a thread or when streaming, the emulsion assumes a striated appearance due to a parallel arrangement of the now elongated and illuminated emulsion globules (Fig. 121a). Under stress, the globules become distorted into rods which are oriented end to end, sometimes so close as to appear to form a continuous thread (Fig. 121b). This structure, first brought out in detail and with strong contrast by the Spierer lens, had been previously revealed, though less distinctly so, by ordinary (light-field)

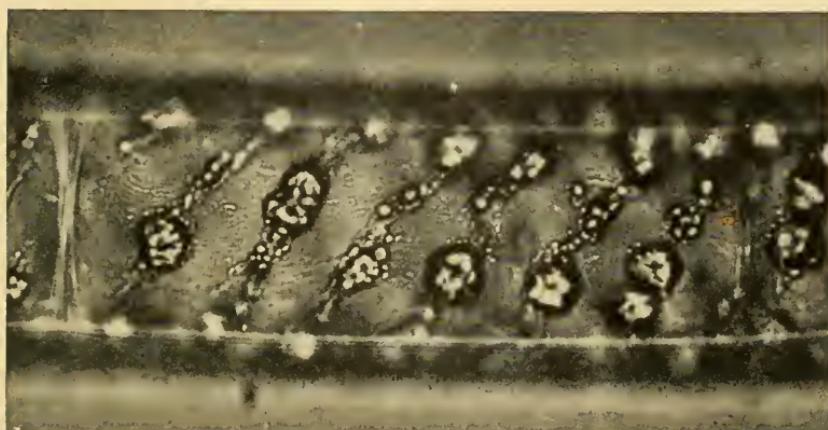


FIG. 122.—The striated structure of the fine protoplasmic emulsion in a Spirogyra cell. Note the delicate lines between the large bands of the spiral chromatophore. The structure of the protoplasm appears to be that of threads, but the threads are broken as in Fig. 119a. (From George Searth.)

methods. A photograph by Searth shows the same structure in the streaming protoplasm of Spirogyra (Fig. 122).

While "dispersed phase" and "dispersion medium" would be sufficient to designate the phases of this emulsion, yet it seems worth while, if for no other reason than to be certain that they will be "definitely reckoned with," to give Greek names to the parts of this delicate visible protoplasmic emulsion. The brightly illuminated dispersed phase of the fine emulsion may be termed *phaneroplasm* (*phaneros*, evident), and the invisible, optically empty background, or continuous phase, *cryptoplasm* (*cryptos*, hidden).

The more closely one approaches the ultimate structure of protoplasm the less easy it is to differentiate vitally between the relative importance of its constituents, but if we attempt to

distinguish between phaneroplasm and cryptoplasm from the viewpoint of their vital significance, then, discontinuity of the former and active streaming of the latter suggest that cryptoplasm, the continuous phase, is the more fundamental of the two.

Fixed Material.—The advent of cytological technique, between the years 1870 and 1890, proved a great impetus to the study of protoplasmic structure and yielded much of value, even though many of the ideas formulated at the time have had to be discarded.

When cells are prepared for microscopic study, they are killed by fixatives (alcohol, formaldehyde, etc.), stained with dyes, and sectioned. Such treatment may result in artifacts—structures not existing in the normal living state—on the other hand, the method may reveal preexisting structures which were not visible in the optically undifferentiated, living protoplasm.

There has long persisted in the minds of biologists the thought that there must exist a continuous framework of some sort which is the structural background of protoplasm. Life in a dispersion (solution) of isolated units, no matter how complex the mixture, is inconceivable. Both this theoretical concept and actual observations on fixed and stained material gave support to the presence in protoplasm of a structure variously described but in each instance consisting of a meshwork or entanglement of fibers, forming a three-dimensional net or sponge. The idea of continuity in protoplasmic structure is thoroughly sound and is supported by ample evidence, though much, yet by no means all, of the cytological support (based on fixed material) given to it is faulty.

The Fibrillar Hypothesis.—The *fibrillar* hypothesis, advanced by Flemming and others, ascribes to protoplasm the structure of an entanglement of fibrils. Flemming elevated these fibrillae, as did Altmann his granules, above the lowly station of mere structural units and viewed them as the seat of the energies on which life depends. The drawings of Flemming of connective tissue, of Heidenhain of muscle and spinal ganglion cells, and preparations of Strasburger (Fig. 123) depict a fibrillar structure. Such a structure is characteristic of and visible in certain living tissues. Ettisch, with the aid of dark-field illumination, finds the construction of sinew to be that of an aggregation of minute fibers (Fig. 124). The fibers of microscopic dimensions are

probably built up of finer ultramicroscopic, invisible fibers, present in living tissue generally.

The Reticular Hypothesis.—Linear structural units may be oriented so as to form an entanglement such as exists in a brush heap, or they may be arranged in a more orderly manner in the fashion of a three-dimensional net. Earlier controversies often centered on the question whether protoplasmic fibers are discontinuous or anastomose to form a net, or *reticulum*. The meshes of the supposed protoplasmic net were said to be from

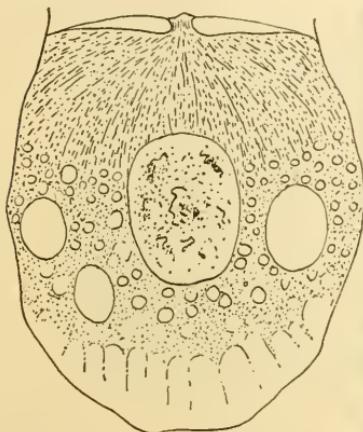


FIG. 123.—The fibrous kinoplasm (above) and emulsion of trophoplasm (below) in an egg of *Marsilia*. (After Haberlandt from Strasburger.)

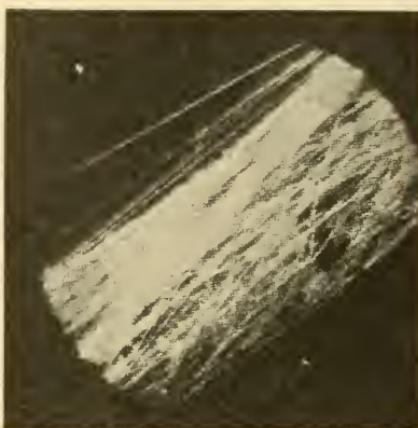


FIG. 124.—The fibrous structure of sinew from the frog as seen with dark-field. (From G. Ettisch.)

$\frac{1}{2}$ to 2μ in size. Whether the purely anatomical framework or the "hyaloplasm" ("enchylema") which bathes it is the real living substance was judged in favor of the latter.

The concept of a reticulum as the structural framework of protoplasm has persisted in medicine in the widely recognized *stroma* of the red blood cell. The stroma is presumed by Bechhold to be a delicate, weblike net, peripherally located. Microdissection studies fail to reveal any such framework either in the large (nucleated) amphibian erythrocyte or in the human corpuscle. The concept is a justifiable one and, as a general hypothesis of the ultramicroscopic structure of protoplasm, finds ample indirect evidence in other material, but there is no visible framework of a substantial nature in the living cell; as for the red blood cell, it is simply a sac (see page 60).

The reticular, fibrillar, net, and spongelike structures seen in fixed protoplasm may be true fibrous coagula or pictures of an emulsion caught in a coagulum; for example, "chromatin granules" on a "linin thread," a structure that has played a prominent role in modern cytological and genetical theories of nuclear behavior, is readily reproduced by a distorted emulsion of large globules with a minimum of dispersion medium. The granules would then be the interstices where several globules approach each other, and the linin thread would be the connecting strands of the dispersion medium. This is shown by comparing a typical drawing of chromatin material (Fig. 125) showing

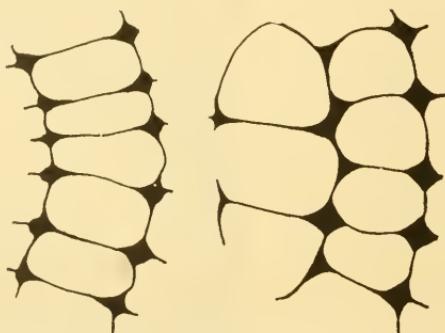


FIG. 125.—A typical cytological picture of "chromatin granules" on a "linin thread." (Compare with C, D, and E, Fig. 150.) (*From C. J. Chamberlain.*)

chromatin granules on a linin thread, with one or more of the possible configurations of an emulsion (particularly E, Fig. 118). The symmetrical arrangement of the phases of an emulsion might well pass for the picture of a net or reticulum.

With the older concept of a framework as the structural basis of protoplasm in mind, but with the realization that the earlier evidence for it is not always sound, let us turn to modern theories.

THE ULTRAMICROSCOPIC STRUCTURE OF PROTOPLASM

The ultramicroscopic structure of protoplasm, like that of nonliving matter, is obviously not visible, but theories pertaining to it, like those of molecular and atomic structure, are based on sound though indirect evidence. We can best approach our problem by a simple analogy. Of two soap solutions, one of low concentration and low viscosity and one of high concen-

tration and high viscosity, the former was elastic and the latter not; the former held a small metal particle in suspension, while the latter could not support the same particle. It would seem, therefore, that the elastic yet thin soap solution possessed a structure that would account for its elastic qualities and for its ability to support a metal particle, while the thicker yet inelastic soap lacked such a structure. This supposition was supported by microscopic examination. The elastic soap solution contained long and slender crystals, while the other soap resembled chalk dust. We have in the behavior and

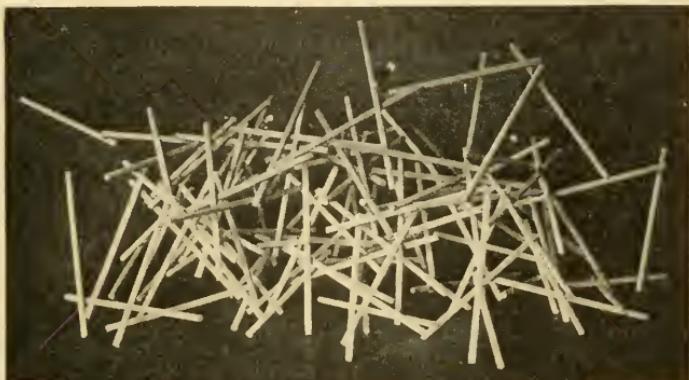


FIG. 126.—A brush heap of imaginary linear molecules.

structure of these two soaps the basis of all generally accepted hypotheses of the structure of jellies. Elastic colloidal systems are built up of linear crystalline units. Their intermeshing gives elasticity and rigidity to liquids which yet flow freely and smoothly. This is structurally possible if we regard the framework of fibers as not fixed but labile, capable of readjustment and comparable to a loosely put together brush heap (Fig. 126). A brush heap is elastic; a sand pile, inelastic.

Before carrying the story of the fibrous structure of protoplasm over to cellulose, investigations on which have yielded much in regard to gel structure in general, let us see how the intermeshed fibrous structure is associated with the protoplasmic emulsion. Milk illustrates the situation almost perfectly. Viewed through the microscope, milk is an emulsion of butterfat in an aqueous medium. More than this is not visible. When milk coagulates, the emulsion plays only a passive part. It is

the casein in milk which coagulates. The fluid whey, an aqueous solution of salts, sugars, etc., separates from the casein coagulum. There are thus in milk three quite distinct systems, intimately associated, *viz.*, an emulsion of fat, a dispersion of fibrous units capable of forming a coagulum, and a solution of salts, etc., permeating the whole. So it is with protoplasm.

Investigations on the structure of cellulose give the best possible insight into modern interpretations of the mechanism underlying the behavior of colloidal jellies, including protoplasm.

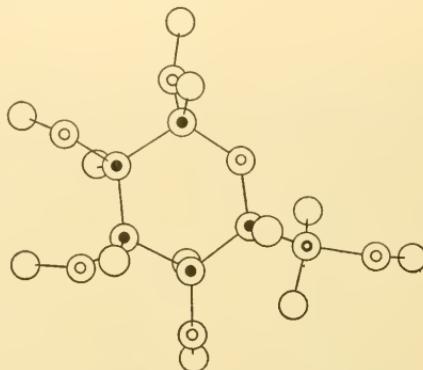


FIG. 127.—Three-dimensional model of a glucose molecule (an amylene oxide glucose unit): circles with black spots are carbon atoms; those with rings are oxygen atoms; the others are hydrogen atoms. (After O. L. Sponsler.)

The Structure of Cellulose.—The cellulose molecule is now thought to be a chain built of rings of anhydrous glucose, $C_6H_{10}O_5$. This latter group has long been known to be the basic unit of cellulose and all higher carbohydrates, but the number and arrangement of the rings in the larger cellulose molecules were not known. Sponsler has suggested the arrangement in Fig. 127, which is of glucose (an amylene oxide glucose unit). The generally accepted configuration for the entire cellulose molecule is that in Fig. 128, where each anhydrous glucose ring is joined to its neighbor by an oxygen bridge, and every alternate ring is the reflected image of the one on each side of it; *i.e.*, it is rotated through 180 degrees. Two such rings constitute an anhydrous molecule of the sugar cellobiose, $C_{12}H_{22}O_{11}$. Some forty or more of these rings, so-called glucose "residues," joined in a continuous chain, form the cellulose molecule.

The linear cellulose molecule has at each end an apparently unsatisfied valence bond. There is little likelihood that such

a free carbon bond actually exists; it rather indicates where our knowledge ends. The bond is possibly satisfied by a univalent (OH) group or attached to an adjoining chain.

The length of the cellulose chain is not fixed. It is capable, stoichiometrically at least, of reaching any length. One cannot, therefore, speak of a cellulose molecule in the strict sense if by molecule is meant a unit of fixed weight and constitution. A length of 40 glucose residues, or twenty times the length of the cellobiose molecule (10.3 A. U.), represents a chain length of about 200 A. U. This is a minimum. Several times this probably more closely represents an average. Staudinger has assumed that the macromolecule of the cotton fiber, which appears to be the longest, may be 1,000 A. U., and E. O. Kraemer has shown (from viscosity and ultracentrifugal determinations) that the cellulose molecule may reach the microscopic dimension of 1.7μ (the diameter remains the same, *viz.*, that of a glucose molecule). Physically, the molecules must be regarded as comparatively stiff threads.

The molecular weight of the average chain molecule has been put at 30,000 to 40,000. Stamm obtained the latter value by centrifuging in a high-speed Svedberg centrifuge (page 477). As the length of the chain varies, the molecular weight will vary; consequently, the much greater length attributed to the cellulose molecule by Kraemer means a greater molecular weight; in fact, the estimated length was based on molecular-weight determinations. The weight now given by Kraemer for the 1.7μ molecule is 500,000.

There are many polymeric materials which are constituted on the same principle as cellulose, in that their molecules are characterized by a chain of recurring structural units; rubber is such a substance.

With this information as a starting point—though it was at the time less precise than now—the problem was carried forward by other X ray workers, who included Scherrer and Herzog. Spectrograms (Fig. 129) indicate clearly that the structure of

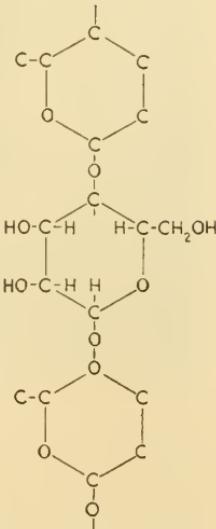


FIG. 128.—Part of a cellulose molecule.

cellulose is symmetrical, that is to say, crystalline. Spectrograms are pictures of diffraction phenomena resulting when X rays are broken up by the lattice-like structure of crystals.

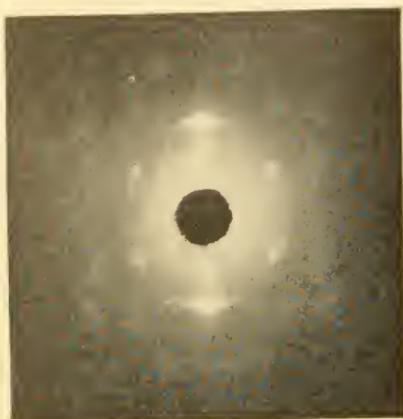


FIG. 129.—X ray spectrogram of cellulose. (From A. W. Kenney and H. Aughey.)

X rays were long thought to be similar to light rays, except that they did not show diffraction. This was due to their very short length. To scatter any type of wave, one must have an obstacle as small as the wave. Diffraction gratings of 1,000 lines to a millimeter (the lines would then be $1\text{ }\mu$ apart) will break up ordinary light waves (of $0.5\text{ }\mu$) but not such minute wave fronts as those of X rays. The German physicist Laue had the brilliant idea that the latticework of atoms which constitutes the structure of crystals (Fig. 143) presents a grating sufficiently fine to disturb X rays. His prediction was verified, and the discovery now serves as a

means for establishing the crystalline nature of substances on the basis of their X ray diffraction patterns. Not only does a symmetrical distribution of bright points on the

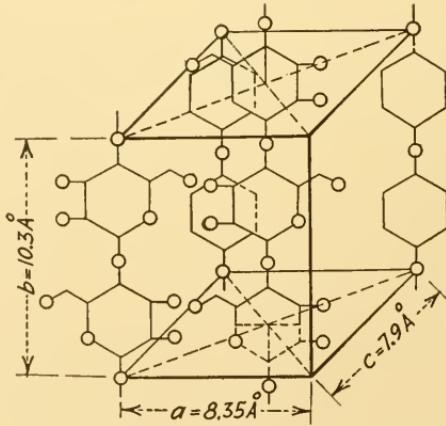


FIG. 130.—The elementary cell of cellulose. (From K. H. Meyer.)

means for establishing the crystalline nature of substances on the basis of their X ray diffraction patterns. Not only does a symmetrical distribution of bright points on the

spectrogram indicate symmetry in the substance photographed, but by studying the distances (identity periods) between these points, the precise arrangement of parts in the crystalline framework can be determined.

It is necessary first to ascertain the so-called *elementary*, or *unit, cell* of a crystal. This space is the smallest parallelopiped within a crystal that still has the properties of the material as a whole (Fig. 130). The number of molecules in the elementary cell of a simple crystal, such as sodium chloride, is four.

In the case of cellulose, it is not molecules but anhydrous glucose groups ($C_6H_{10}O_5$) that build up the elementary cell to the number of four.

The unit cell of cellulose has the dimensions 8.35 A. U. \times 10.3 A. U. \times 7.9 A. U. The length, 10.3 A. U., is the equivalent of two anhydrous glucose groups of which 40 to 100 or more constitute a chain. A model of a unit cell would preferably contain 10 such ($C_6H_{10}O_5$) groups, *i.e.*, that part of Fig. 131 contained within the heavy lines forming a cube; but as each unit cell when entirely surrounded by others shares each vertical edge with three other unit cells, then the number of anhydrous glucose groups that can be allocated to each individual unit cell is four, *i.e.*, two on one edge and two on the center axis which are not shared. Because of the small number of radicals contained within an elementary cell, early work on cellulose led to the belief that the cellulose molecule was of low molecular weight; but the elementary cell determines the character of the crystal and not necessarily that of the molecule. Organic chemists found it very hard to reconcile the known properties of cellulose with the idea of a low molecular weight. The long chain molecule was therefore postulated.

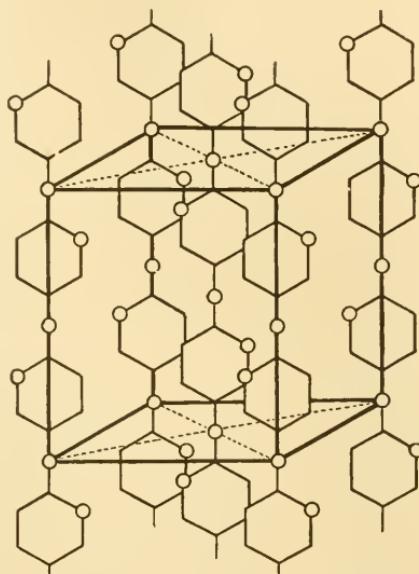


FIG. 131.—Part of a cellulose micelle or crystallite.

In the case of common salt, there is a repetition of the unit cell in all directions, giving a homogeneous distribution throughout the crystal. But in cellulose, as is likely to be true in colloidal material generally, the distribution, of unit cells and of molecules, is not homogeneous; instead, the long chains are

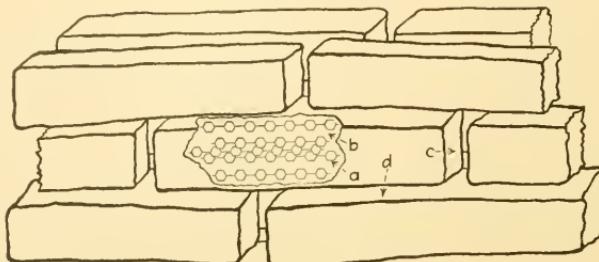


FIG. 132.—Orientation of micelles in a block of cellulose.

aggregated into bundles of some sixty chains each. These bundles, being molecular aggregates, satisfy the botanist Nägeli's definition of a *micelle*. We shall recall (page 118) that Nägeli postulated a so-called micellar structure for all gels, including

protoplasm, the unit of the structure being a micelle, or aggregate of molecules, *i.e.*, a colloidal particle. As the cellulose micelle is symmetrical in structure and therefore crystalline, it has received the name of *crystallite*. An association of cellulose crystallites, oriented much as are bricks in a wall, presumably constitutes the colloidal structure of cellulose (Fig. 132).

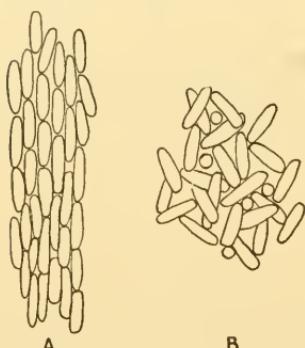


FIG. 133.—Orientation of micelles in (A) ramie and (B) cellophane. (After H. H. Mark.)

The precise orientation of the micelles is of significance in such properties as conductance and tensile strength. H. H. Mark depicts two extremes—one in which there is perfect parallelism, and the other in which there is a random or brush-heap distribution of the micelles, the former represented by native ramie, and the latter by cellophane (Fig. 133). The cellulose of flax displays an excellent orientation of micelles parallel to the fiber axis and has a tensile strength comparable to that of the best steel. The following table (from Mark)

shows how well tensile-strength values agree with X ray analyses of symmetry:

Material	Kg./sq. mm.	Material	Kg./sq. mm.	Material	Kg./sq. mm.
Steel.....	170	Silk.....	35	Rayon, ordinary.	18 to 20
Copper.....	40	Cotton.....	28	Rayon, oriented	60
Lead.....	3	Flax.....	Over 100	Rubber, ordinary	15 to 20
				Rubber, oriented	60

Mark adds another possibility, *viz.*, that of pronounced overlapping of the molecules of one bundle with those of another (Fig. 134A), a very likely condition in that the molecular chains of a cellulose micelle are of different lengths. Such an arrangement would provide maximum strength in the direction of the

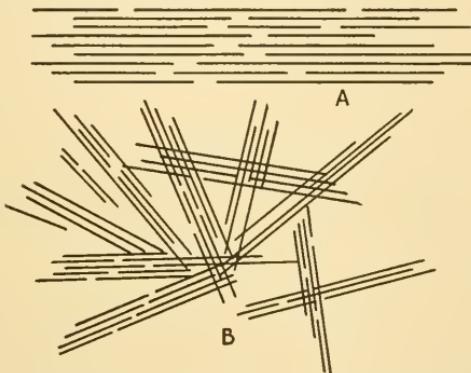


FIG. 134.—A, orientation of molecular chains in cellulose, orderly and parallel to the fiber axis, but terminal boundaries irregular; B, random arrangement of molecular aggregates. (After Carothers.)

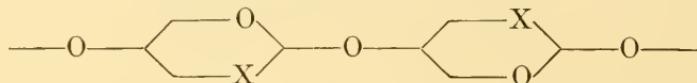
fiber axis, because the mutual cohesive force of the long chains would be fully utilized. In regeneration cellulose (cellophane), says Carothers, there is random orientation (Fig. 134B). The molecules are brought into an ordered arrangement by mechanical stress. The strength of a sheet of cellophane which is initially the same in all directions can be so changed by stretching that its strength along the axis of stretch is increased several times.

If we turn for a moment to other substances of an organic nature which have been subjected to X ray study and found to be crystalline in nature, with linear units in often orderly

arrangement, we find that the list is a long one; it includes starch, gelatin, chitin, rubber, silk, hair, keratin, sinew, muscle, nerve, and brain. It is but a step from these to protoplasm—indeed, muscle, nerve, and brain are protoplasm.

Not all organic substances that yield spectrograms do so with equal clarity. Herzog finds that, in sharpness, the X ray interference of hair is but fair, sinew better, and silk still better; muscle and nerve are less clear yet indicate crystalline character. None of the pictures of these substances can be analyzed with the accuracy of those of the best crystals, yet all show fibrous structure with crystalline symmetry, only less perfect, as is to be expected of colloidal matter.

The case of chitin is particularly interesting. Chitin (a poly-acetylglucosamine of both plant and animal origin) is said to be the most chemically resistant skeletal material known. Its resemblance to cellulose is expressed by the formula



where X equals OH in cellulose, NHCOCH_3 in chitin, and NH_2 in chitosan. Herzog found that the chitin plate of the crab gives an X ray picture identical with that of sinew in spite of a different chemical constitution. This is true because the chitinous shell in Crustacea is a direct transformation of sinew from which it arose by a slow change. It is completely converted chemically but unchanged structurally. Substances so transformed Freundlich calls permutooids.

X ray studies on nerve have been carried out by G. L. Clark, F. O. Schmitt, and J. N. Mrgudich. They find that the molecular configuration producing the diagram in nerve is probably due to a system of oriented protein primary valence chains lying parallel to the fiber axis. The equatorial spacing of 17 A. U. corresponds to the direction of the side chains, and the meridional spacing may correspond to the reflection from double amino-acids residues along the fiber axis. The radiation required to produce these patterns has no appreciable effect on the irritability of the nerves. Nerve, then, is essentially a single system of partially oriented primary valence chains probably admixed with unoriented intermicellar protein chains.

Polarization studies on the cell walls of plants have been made by Frey-Wyssling. The method is that of immersion in liquids of known index of refraction. When a crystal is immersed in a liquid the index of refraction of which is equal to that of one of the three indices of the crystal, then the boundary between liquid and crystal, viewed in the light furnished by Nicol prisms, disappears when the plane of polarized light is in line with the direction of that particular crystal index which is identical with the index of the oil. A series of mixtures of amyl-benzyl alcohol and cinnamon clove oils of increasing refractive indices were used. In this way, Frey-Wyssling established the three main indices of refraction $n\alpha$, $n\beta$, and $n\gamma$ (Fig. 135). By these means, he showed that in cell walls there are submicroscopical (colloidal) rod-shaped particles which he identifies with the Nägeli micelles. The long axis of the micelles corresponds to the direction of the greatest refractive index; the latter value, therefore, gives the orientation of the micelles in the wall.

Photographs of cellulose taken with the Spierer lens (page 98) add further evidence to the general conclusion that cellulose possesses a colloidal structure of symmetrically arranged rods. The lens reveals parallel striae (Fig. 136A) which appear to be composed of microscopic units oriented end to end. These units are about 2μ long and were consequently termed *super-micellae* to distinguish them from the ultramicroscopic micellae postulated by Nägeli and now generally accepted by chemists as the unit of colloidal structure. But as the cellulose molecule apparently reaches the microscopic length of 1.7μ , then the supermicellae become micellae in the strict sense, for the latter by definition are bundles of molecules; both must, therefore, be of the same length.

The striae (Fig. 136A) which are built of rod-shaped super-micellae oriented end to end in continuous or discontinuous lines are in parallel arrangement and thus form lamellae, or plates, which in their turn combine to produce the mass of

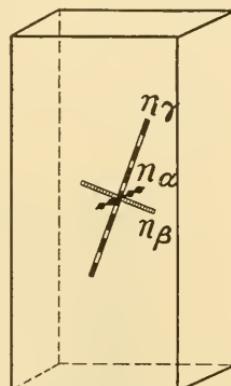


FIG. 135.—Orientation of the indices of refraction in plant-cell walls. (From Frey-Wyssling.)

cellulose. The same striated and articulate structure of cellulose persists in bituminous coal, as shown in Spierer photographs by Thiessen (Fig. 136B). The fibers in wood are also built up of the fibrillae characteristic of plant cellulose in general.

When beaten wood (paper) pulp is examined under the microscope, it is seen to consist of many parallel fibrillae which can be dissected out with the aid of microneedles.

Natural cellulose thus consists of units of ever increasing size, all of which, from chain molecules to visible wood fibers, are of linear form. The orientation of these units determines the physical properties (elasticity, tensile strength, etc.) of the

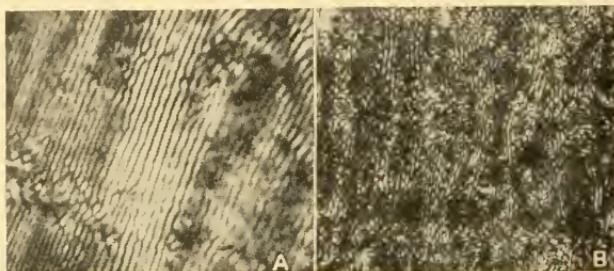


FIG. 136.—*A*, the striated structure of the cellulose wall of plant cells as revealed by the Spierer lens; *B*, similar structure of bituminous coal seen with the Spierer lens. (From H. R. Thiessen.)

material. Where the orientation is orderly and parallel to the fiber axis, the tensile strength is high. Lack of symmetry in arrangement means low tensile strength. The force responsible for orientation may be the purely mechanical one of strain, as in rubber, which unstretched shows no X ray diffraction pattern but stretched becomes crystalline; or the orientation may be due to polarity, a very prevalent property of matter.

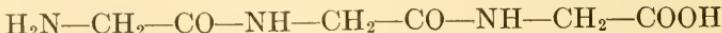
Polarity.—On a number of occasions we have seen that polarity is a property involved in many reactions, for example, it determines the orientation of (polar) molecules at surfaces (page 130). Probably no other force in nature is so widely distributed and plays so great a role in the behavior of systems, from molecules to organisms, as polarity. The term expresses any situation where the two ends or sides of an object are different. A willow cutting shows its polarity when it grows roots at one end and leaves at the other. A molecule is polar if its ends differ. Strictly, polarity should be limited to objects the ends of which

differ in their physicochemical properties. Kinds of polarity may be classified as, (1) *atomic*, where there is a loss or gain of one or more electrons by an atom (sometimes resulting in a *polar bond*, as in primary valence); (2) *chemical*, where there are two radicals, one acid and one basic, at opposite ends (as in the amino acid molecule, $\text{NH}_2-\text{R}-\text{COOH}$); (3) *electrical*, where there are two unlike poles, one positive and one negative; and (4) *magnetic*, where there are also two unlike poles but purely magnetic in character, one north and one south. (The classification is arbitrary and simply a matter of convenience.)

The importance of polarity has long been recognized but is only now beginning to be widely applied. In the living world, it is manifest everywhere. The salt concentration, acidity, electric potential, and metabolic activities of organisms as a whole and of individual cells differ in different regions and usually in such a way as to give a gradient. Votchal and Lund have established electrical potential gradients in spruce and pine trees. Child found metabolic gradients in very lowly animals such as planarian worms and Amoeba. Tissues show electric polarity, *i.e.*, a drop in potential, between them. Cellular polarity is described by F. Weber in that all cells of a tissue plasmolyze at one and the same end. S. Prát of Prague tells of polarity in the vacuoles of cells as determined by staining reactions. The behavior of organisms is to a great extent influenced by their polar properties. Our problem at present has to do with the part that polar molecules play in determining the structure of protoplasm. We can say so little that is direct and definite in regard to protoplasmic structure that we must approach our problem of the role of polarity in the structure of a living system by first considering it in nonliving systems. The part that polar molecules play in the stabilization of emulsions by monomolecular films (Fig. 86, page 130) and in determining the permeability of the plasma membrane and the iridescence of soap films has already been discussed (page 129).

Given long and polar molecules, *i.e.*, molecules with ends unlike as to electrical sign or as to acid and basic properties, it is possible to picture their orientation in mass and to obtain certain types of structure which presumably are typical of gels and at least have the virtue of giving a mechanical basis upon which to interpret the behavior of gels and of protoplasm.

Molecular chains that have an unsatisfied valence bond at each end may readily join, one to another. Linear protein molecules may show such an affinity for each other because of ionization at their unlike ends. A polypeptid illustrates the type of linear and polar protein molecule likely to occur in protoplasm:



Ionization of the terminal groups (NH_2 and COOH into NH_3^+ and COO^-) leaves the ends free to unite. Where there exist unsatisfied valences, as in the cellulose molecules, or ionized radicals, as in proteins, the ends may become saturated (*e.g.*,

with OH radicals), or the molecule may form a closed ring (page 485), or one chain may join to another and form a continuous intertwining thread (Fig. 137). J. A. Wilson regards a similar structure as probably occurring in gelatin. He says that we may look upon a plate of gelatin as a continuous network of chains of amino acids, there being no individual molecules, unless one wishes to regard the entire plate of gelatin as one huge molecule. Another possible picture, and one which better satisfies certain physical properties of lyophilic systems

FIG. 137.—An entanglement of a continuous thread, illustrating a possible structure of jellies. (From V. Cofman.)

such as gelatin, is the following. If the structural configuration of protein molecules is that of long chains with lateral, rather than, or in addition to, terminal unsatisfied bonds (as shown on page 475), then we have possibilities of weak unions along the chain. Such a situation meets the structural requirements of a brush heap of loose construction.

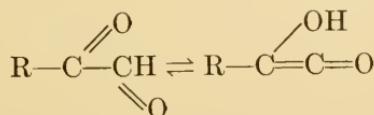
A brush heap (Fig. 126) is, as already suggested, a mechanism permitting of a satisfactory interpretation of the properties of jellies—lyophilic colloidal systems which, whether of high or of very low viscosity, possess elastic qualities. The brush heap is likewise a suitable mechanical basis upon which to interpret the thixotropic properties of certain gels which, though they may contain but little solid matter, set to a firm mass (page 149). Long structural units permit the formation of an open yet rigid



framework out of little solid matter. Gels of iron oxide and calcium germanate, containing but 0.1 per cent of solid matter and, therefore, 99.9 per cent of water, are examples of thixotropic systems. Thin soap solutions, egg white, and the jellyfish are examples of highly elastic systems of low solid content. Protoplasm is both thixotropic and highly elastic.

The brush heap alone satisfies elastic and rigid qualities in systems of high water content, but the thixotropy (of gels which are readily broken down by mechanical disturbance and equally readily build themselves up again) and the fluidity of elastic systems require the additional quality of loose bonds between the fibrous molecules so as to permit ready readjustment.

Evidence that loose bonds may occur along the fibers of elastic systems (*e.g.*, at the points of contact of the imaginary linear molecules in Fig. 126) and thus allow for a mechanical interpretation of fluidity is to be had from examples of tautomeric slips. One such is the change in ionization (and therefore in bonds) known to occur in protein solutions with change in pH. (This instance is less applicable to the problem in hand, as there are apparently no changes in pH to account for readjustment in structure when an elastic solution flows or when a thixotropic gel solvates or gelates.) Another example is the case of internal salt formation in proteins where amphoteric chain molecules of the type of amino acids are converted into rings by the acid and basic radicals mutually satisfying each other; in this manner, the active linear molecule of glycine becomes an inactive ring (page 485). Still another instance of a tautomeric slip which illustrates the ease with which bonds may be shifted is that from the keto to the enol form of glyoxal and back again:



If we turn from these more complex instances of tautomeric shifts to the very simple case of water, we have an excellent example of structural continuity, due to polarity, in a liquid that flows freely owing to constant and ready tautomeric slipping between its molecules. The water molecule is polar; *i.e.*, it has an electric moment (Fig. 138). It possesses, therefore, the

characteristics necessary for molecular orientation. The molecule has a positive and a negative charge; the former is on the two hydrogen atoms, and the latter on the oxygen atom. At a distance, such a molecule is electrically neutral; but viewed nearby, it is positive or negative depending on the position. The negative oxygen atom of one water molecule would attract and hold the two positive hydrogen atoms of another water molecule close by and with a force which may equal its hold on its own two hydrogen atoms. We may regard the valence bonds within a water molecule as chemical and those between adjoining molecules as physical, but it amounts to essentially the same thing in the end, for the hydrogen atoms and the oxygen

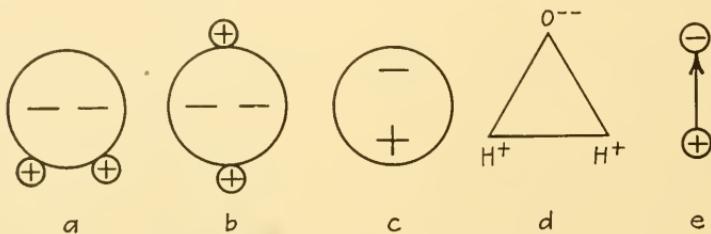


FIG. 138.—Diagrams illustrating the polarity of water.

atoms exert forces on the surrounding molecules which are no less chemical in nature than those holding the atoms within the molecule together. Fluidity depends upon the tautomeric shifts that take place between one water molecule and another. (As water is slightly ionized, the hydrogen nuclei are able to shift to some extent from one water molecule to another, but fluidity does not necessarily depend upon this.) Bragg states that water molecules are always in partnership but always changing partners. Even liquids in solution yield diffraction patterns indicative of some kind of arrangement. We thus see how polarity leads to orientation and it in turn to continuity in structure in a liquid system and in so simple a one as water.

The possibilities in proteins are infinitely greater. A theory of the structure of elastic gels, wherein the gel framework is assumed to be a loosely joined, semirigid, elastic framework built of long, slender, and crystalline fibers, satisfies not only the known properties of jellies but also those of protoplasm, as far as our present knowledge goes. Such a theory makes particularly clear how it is that protoplasm can exhibit properties so typical

of fluids (streaming, rounding up, etc.) and yet also possess properties so essentially characteristic of solids (elasticity, etc.).

The hypothesis of gel structure so far presented is now generally held by the chemists and rapidly winning favor with the biologists as an interpretation of protoplasmic structure. H. R. Procter regards gelatin jellies as built of a network of molecules or of linear aggregates (threads) of molecules. J. A. Wilson is of a similar opinion, though limiting the structural fibers to units of atomic diameter (*i.e.*, a single molecule) or at least comparatively small polymerized groups. The long chains of amino acids satisfy all necessary conditions for such fibers. As already stated, the terminal acid groups may unite with the basic ones until a continuous network is formed. A block of jelly would then become a single molecule, after the manner of crystals. R. H. Bogue pictures the process of gelation from a hot solution (of gelatin) to a solid jelly as involving the formation of catenary threads by the union of individual molecules end to end. The production of soap curds is attributed by McBain to similar units and forces. He ascribes the elasticity of curds to a fine, filamentous structure.

Structural Continuity in Protoplasm.—Having accepted the work of chemists in regard to the structure of gels, and having found this structure to be one of linear crystalline units forming either a brush heap of fibers or a symmetrical arrangement of fibers oriented end to end in parallel rows, our next task is to see how far this hypothesis of structure will hold for protoplasm. That it meets certain qualifications has already been indicated, but we can now give more substantial evidence.

Fibrous structures often appear in cells when these are fixed (killed) and stained (Fig. 123); they must have arisen through aggregation of smaller fibrous units. But we need not go to

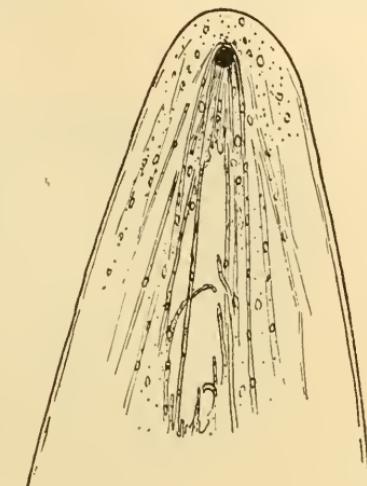


FIG. 139.—Protoplasm being torn by a microneedle (the large black spot) and thereby showing its fibrous structure and the formation of highly elastic strands.

fixed material for indications of a fibrous structure in protoplasm. When the living plasmodium of a slime mold is stretched, the protoplasm (when of sufficiently high viscosity, as it usually is) tears somewhat after the manner of bread dough. Strands within it are conspicuous; as they separate out, they exhibit considerable tensile strength and ultimately snap with suddenness (Fig. 139).

If we turn from protoplasmic qualities to those of tissues, we find again that living matter is built of fibers. The structure

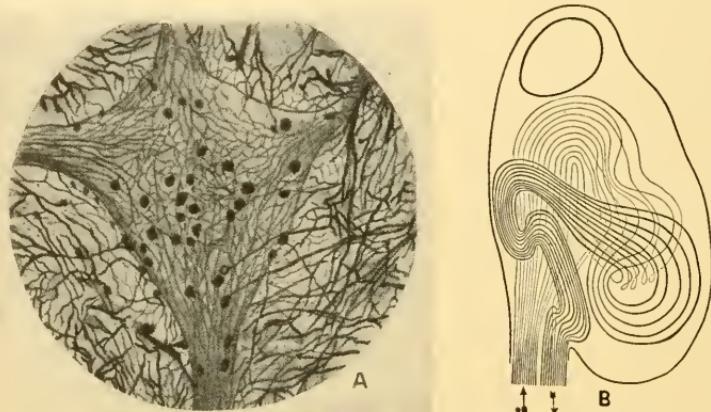


FIG. 140.—A, fibrous entanglement surrounding a nerve cell (*from T. Péterfi after Tscherñjachiwsky*); B, course of the neurofibrils in a spinal ganglion cell of the frog, as graphically reconstructed by Heidenhain.

of muscle is fibrous. Nerve tissue is a bundle of threads (Fig. 140). The high tensile strength of sinew is an indication of its fibrous character (Fig. 124).

Certain behavior phenomena are just as significant in indicating a fibrous or brush-heap structure of protoplasm as are visible proofs. Pronounced and exceedingly sudden changes in the viscosity of protoplasm may occur when the living substance is disturbed by a microneedle. A striking case of this is that already cited (page 151) in which a cell, in mid-mitosis, with its intricate figure of spindle and astral rays, was penetrated by a needle, with the result that the entire mitotic figure immediately collapsed, leaving only a relatively homogeneous mass of protoplasm, with no sign of the previously existing cell structures. The collapse is a perfect example of thixotropic behavior. Changes in the viscosity of protoplasm due to

agitation with a dissection needle, less sudden and pronounced than that just cited, are common. These phenomena are all duplicated in colloidal systems. Thixotropic behavior in gels is now a commonly known happening. W. Ramsden gives another case which is comparable. He tells of pouring a dilute filtered solution of white of egg repeatedly to and fro from one test tube into another or vigorously shaking it in a closed vessel, with the result that numerous loose fibrin-like flakes develop in the liquid. They consist of agglutinated protein and are permanently insoluble in the mother liquid. It is even possible by prolonged shaking to convert the whole of the protein into an insoluble solid. Protein molecules are simply aggregated into visible masses of solid, but no coagulation has taken place. The viscosity of gelatin solutions is lowered by agitation alone, which is evidence in favor of a structure such as that postulated here. The same viscosity changes in protoplasm from the same causes rest on the same structural features.

That the mitotic figure (Fig. 17), including the much controverted spindle fibers, owes its existence to structural features resting upon the orientation of linear crystalline units seems very likely, especially in view of Zocher's observations. He saw the parallel orientation of rod-shaped particles in a vanadium pentoxide sol; doubly refractive (crystalline) images resulted which greatly resembled the mitotic spindle of cells (of eggs immediately after fertilization). Bělař, Freundlich, Runnström, Spek, and others regard the mitotic figure as the outcome of an orientation of fibers.

Further support for linear units in protoplasm comes from a number of diverse observations; thus, A. R. Moore finds that plasmodia when forced through moderately fine sieves do not live; but they may of themselves flow through exceedingly fine sieves. Forcing presumably crushes the long protoplasmic fibers, but in flowing naturally the protoplasm can take its fibers through much finer pores. Moore believes the microfibrils to be of the order of 5×10^{-5} mm. in diameter and two thousand times as long. Peters has postulated similar but finer molecular threads in protoplasm.

It is of further significance for the problem of protoplasmic structure to realize that while polarization studies of protoplasm have not shown living matter generally to be anisotropic, yet

they have shown striated muscle and types of connective tissue to be so; and Scarth has demonstrated anisotropic qualities of chlorophyll, a common constituent of certain plant cells. Furthermore, muscle, nerve, and brain (which are protoplasm) have yielded spectrograms (X ray diffraction patterns), as have sinew, hair, silk, etc., indicative of a crystalline nature.

The possible significance of cytoplasmic structure in physiological behavior is indicated by A. R. Moore, who finds that neither sperm nor egg nucleus (of echinoderms) has any effect on segmentation tempo, the reactions of the cytoplasm alone determining it.

Cellular Organization.—Whatever life may be, and however much we may try to explain it on the basis of relatively simple physical phenomena, there always remains that greatest of all bodily and protoplasmic qualities, *organization*. Fully to interpret protoplasmic organization in physical terms is impossible; it is too intricate—it is life itself. But we can point to several physical properties which are a part of and therefore to a degree determine cellular organization.

How is it possible for protoplasm to carry on so many different processes simultaneously without one interfering with the other, all within the confines of a single cell? This is one of the oldest of questions in cellular biology. It may be answered by the supposition that delicate and temporary membranes, consisting of nothing more than firm protoplasm, traverse the cell in all directions. An excellent example of this is to be had in a myxomycete plasmodium, where temporary channels or arteries of protoplasmic flow are set up. These arteries guide the protoplasm along definite routes which are broken down and reestablished as the plasmodium progresses. The streaming protoplasm does not pass beyond the ephemeral boundaries of the arteries, though the latter are also of protoplasm. Their formation and temporary maintenance are undoubtedly made possible by a structural (fibrous) framework which endows the membranes with the required degree of rigidity.

The concept of continuity in structure, so necessary as a mechanical basis for the interpretation of the physical nature of protoplasm, is opposed by those who regard protoplasm as essentially a solution. They must therefore also discard that most fundamental of all vital qualities, organization. The

fluidity and assumed water miscibility of protoplasm are usually cited in support of the idea that protoplasm is a pure solution. Liquids flow freely and round up readily into droplets. As protoplasm does the same, it is assumed that it must be a pure liquid or a true solution throughout. But we have seen how fluidity is also characteristic of gel-forming systems which show elastic qualities even when quite thin.

Belief in the water miscibility of protoplasm dates back many years, though its discoverer denied that such a property characterized it. Dujardin described protoplasm as "immiscible in water." The opposing point of view rests upon a misconception. Those who believe that protoplasm mixes freely in water maintain that it is ordinarily kept from doing so by the presence of a membrane—an outer oily layer. Protoplasm, when cut or torn, immediately forms a new surface over the wounded part, if it is to remain alive. That a protective membrane was there and is again formed over the wound is certainly true, but torn protoplasmic surfaces may remain ragged and exposed for some time. There may then be no indication of miscibility at the unprotected surface until a complete breakdown occurs. At death, the protoplasm quickly diffuses into the water or coagulates.

The colloid chemist readily grasps the idea of a jelly liquid enough to flow yet possessing gel (solid) qualities. He can also visualize a system like gelatin which takes up water with avidity and yet gives no indication of miscibility with it (when cold). Water enters by imbibition in gelatin and in protoplasm. Miscibility is prevented by structural continuity which holds the gel together though permitting swelling. Loose contact between the structural units allows for readjustment, but the bonds are too strong to sever completely, unless, as in the case of gelatin, freed by heat.

The osmotic properties of protoplasm appear to be other evidence in support of the idea that protoplasm is a true solution comparable to salt in water. Heretofore, it has been customary to refer to the taking up of water by protoplasm as imbibition rather than osmosis. In plants, osmosis is the predominating force involved in water intake because of the large cell vacuole. In animal cells, the situation was thought to be the reverse, imbibition predominating. But the researches of Lucké indicate

that an animal cell (echinoderm egg) obeys the laws of osmosis. This would imply that the cell is a sac containing a solution.

When water is injected into a cell, it often perfuses the protoplasm quickly. This is no more miscibility than the taking up of more water by a partially filled sponge. When a sponge, a block of gelatin, or protoplasm reaches its maximum capacity to hold water, it takes up no more. This is not true of solutions, except when confined in an osmotic sac (which is the viewpoint of the opponents of protoplasmic organization).

Whether we turn to newer work on the physiology of the cell or to older work in cytology, we find support for a semirigid framework in protoplasm. Scarth has demonstrated "a supporting skeleton" and a matrix in protoplasm, adding further that cytoplasm is characteristically elastic, that the impression of fluidity is illusory, and that the architectural features of a cell give a structural basis for organization. Spek corroborates this observation.

The older workers in cytology held similar opinions, expressed in the "spongioplasm" (framework) and "hyaloplasm" (intervening fluid) of Leydig and the "ground substance" and "reticulum" of Carnoy and others. E. B. Wilson states that the "continuous substance" (*i.e.*, spongioplasm) is the most constant and active element and that which forms the fundamental basis of the protoplasmic system, to which E. G. Conklin agrees in saying that protoplasm is composed of a more fluid and a more viscid portion. He bases his statement on experiments in centrifuging the eggs of *Crepidula*, where he found that the more fluid portion of protoplasm may be readily moved but the more viscid portion not so readily; also, the more viscid part of the protoplasm holds the nucleus in place in definite relation to the periphery of the cell and brings parts back to their normal positions when once they have been displaced by centrifuging. Conklin concludes that the polarity and general organization of the egg reside in the more viscid substance and not in the more fluid medium.

Alsberg, writing nearly twenty-five years ago, said, "A study merely of chemical constitution, however necessary, will carry us but a very little way in understanding even the simplest processes which take place in protoplasm, unless it is combined

with a study of structure and of the dynamics resulting from both."

Cellular organization rests upon structural continuity, just as the purely mechanical properties of protoplasm rest upon it. Without both, it is impossible to account for the multiplicity of reactions simultaneously going on within the confines of a single cell. The harmonious functioning of a cell is but another name for life. The one and therefore the other are dependent upon the structural continuity of protoplasm.

CHAPTER XVI

PERMEABILITY AND THE PROTOPLASMIC MEMBRANE

Problems in science have their day, but there is one biological problem that has held a foremost position since its inception nearly a century ago. That problem is *permeability*. It has held its position because of the mystery that surrounds it and because it is a protoplasmic quality of prime significance to the cell.

Just as an osmotic system (page 183) owes its capacity to develop pressure to the selective permeability of a membrane which permits water but not dissolved substances to pass through freely, so also does the living cell owe this and other properties to the selective permeability of the protoplasmic membrane. To this quality does protoplasm owe its extraordinary capacity to determine the entrance and exit of substances into and out of the cell. The capacity of protoplasm to select is known as *selective*, or *differential permeability*. The more commonly used term *semipermeability* originally referred to the full permeability of the membrane for water and its apparent impermeability for dissolved substances. The expression is now used in the same sense as selective permeability. Cell permeability control is presumed to lie primarily in the outer plasma membrane.

The capacity of the cell to select substances is presumed to lie primarily in the protoplasmic membrane; the surface layer is certainly the first barrier that must be passed. Permeability problems, therefore, assume two aspects—that dealing with the question of what substances enter and that having to do with the nature of the membrane which gives to it its capacity to select.

PERMEABILITY

There are four major methods of ascertaining the means by which substances enter a cell—the visual method, by direct

observation of the entrance of the substance, *e.g.*, a dye; the osmotic method, in which the rate of plasmolysis and ease with which a cell regains its original distended form are criteria of the rate of entrance of external salts; the chemical method, through analysis of the cell contents; and the electric method, in which the conductivity of the cell sap is measured in relation to the external solution. The last two methods are the most accurate, though they offer difficulties enough. Chemical analyses can be satisfactorily made of cell sap only; the protoplasm itself undergoes many changes at death which make it impossible to know what was there when the cell was alive. Cells which have very large central vacuoles, as has the coenocytic alga *Halicystis*, are excellent material for chemical analyses of the sap.

One of the first facts definitely known about the permeability of the cell was that substances, such as ether, alcohol, chloroform,

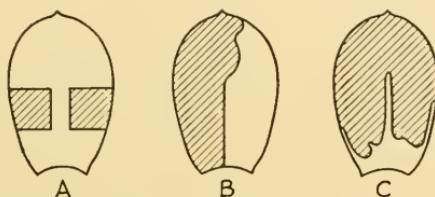


FIG. 141.—Diagrammatic sketches indicating those regions of an *Elodea* leaf which first succumb to the toxic effect of ethyl alcohol: *A*, after brief treatment; *B*, after longer treatment; *C*, after one hour.

methane, and xylene, which dissolve fats, enter with extreme rapidity, while salts enter very slowly. The German botanist Overton reasoned that if fat solvents enter readily while salts do not, then the protoplasmic membrane must be fatty or lipoid in nature. That the outer layer can not be pure lipoid, particularly cholesterol, is obvious, otherwise water-soluble substances such as salts, so necessary to organisms, could not enter. (Water-soluble substances would pass through a hydrophilic lipoid such as lecithin much more readily than through a hydrophobic substance such as cholesterol.)

As organisms require salts, these must of course enter cells, but it was early learned that they enter very slowly and not all at the same rate. This latter statement is also true of the fat solvents, which ordinarily enter so rapidly; thus, cells within the same

leaf—indeed, adjoining cells—permit alcohol to enter at quite different rates. A block of cells within a certain area may be killed by 10 per cent alcohol within a few seconds, while immediately adjoining cells survive for some minutes (Fig. 141).

Many studies have been made on the rate of entrance of salts of the common metals into cells. These all lead to the conclusion that a definite series of cations exists, of which the following is an example:



in which rubidium enters the cell most rapidly, and calcium the least rapidly. Among anions, it seems that the nitrate ion enters faster than the chlorine ion, which is somewhat faster than the sulphate ion. While there are theories, to be taken up shortly, which satisfy certain permeability phenomena very well, there is no adequate explanation for the selective permeability of ions. In the above series, the ions fall into two groups, the members of each group being alike as to magnitude of charge and valence. One can do little more than list the observed facts.

A number of so-called permeability phenomena may not, in truth, be such, at least in their entirety. We must first distinguish between passive and active permeability. Strictly speaking, only the former is true permeability; the latter is cellular activity. It is known that certain living (the apple skin) and nonliving (celloidin) membranes are more permeable to the cation potassium than to the anion chlorine. This may be a strict permeability phenomenon, or it may be one of electrokinetics in which electric potentials are involved, as in a galvanic cell, where anions travel in one direction and cations in another. A porous membrane inserted between the two need not affect the results.

The lining of the human intestine is readily permeable to water and other substances, while the stomach absorbs little or nothing of either. We may here be dealing not with permeability processes as ordinarily interpreted but with the adsorption of water by tissues. Tissue permeability is quite a different thing from cellular permeability, because in the former case much is taken in as *intracellular* matter. Tissue, like that of the intestines, is permeable to all electrolytes; *i.e.*, it is not semi- or selectively permeable. Certain other types of animal

tissue exhibit true cellular permeability. This is true of the kidney, the tubules of which function in their permeability like that of a typical cell. There is also the possibility that tissue such as that of the lining of the intestines may owe its permeability to purely mechanical processes. For example, the intestines and other living membranes permit water to pass through more rapidly in one direction than in another. This may not be a true permeability process at all but due to certain cellular activities, such as rhythmic contraction of the intestinal villi.

Another so-called permeability phenomenon which has aroused considerable interest and discussion, and which may not be a permeability process at all but one of metabolism, is that of the relative rate of entrance of different sugars into the cell; thus, it is generally known that glucose enters cells more readily than does sucrose. Further, glucose is fully absorbed in the body, while fructose and manose are not. It is further said that the dextrorotary form of certain substances (alanin) is taken up by the animal body, while the levorotary form is not absorbed and appears in the urine.

Attempts have been made to explain these phenomena in terms of strict permeability processes—*e.g.*, on the basis of size of molecule. The whole problem of sugar absorption appears to be primarily, if not solely, one of metabolism. Glucose is used in respiration, while sucrose is not. Consequently, as glucose is destroyed within the cell, there is room for more, strictly on the basis of a concentration equilibrium. As the sucrose is not used, no more enters. The protoplasmic membrane may thus play no part whatever in determining the rate of entrance of sugars into the cell, metabolic processes alone being responsible. This may also be true for the little understood passage of sugar from one cell to another, in which sugar enters a cell at one end and leaves it at another.

In contrast to the foregoing phenomena, others equally difficult of interpretation rest apparently upon selective permeability pure and simple; such is the more rapid entrance of sodium chloride than that of sodium sulphate into cells.

Selectiveness is the outstanding characteristic of cell permeability; next in significance is change. The permeability of the plasma membrane is not a fixed property. Change in perme-

ability is a normal feature of cellular activity. It may also be brought on by a change in environment.

Experimental results on the effect of salts on cell permeability are contradictory, but one fact seems to be rather generally true, *viz.*, that sodium increases the permeability of the protoplasmic membrane, and calcium decreases it. Even this conclusion has been found not to hold at all times; thus, the American physiologist S. C. Brooks found that sodium chloride, potassium chloride, calcium chloride, and magnesium chloride all decrease the rate of penetration of a dye into the living cell; *i.e.*, they lessen the permeability of the protoplasmic membrane. That sodium increases and calcium decreases permeability agrees well with the disintegrating (solation) effect of sodium and the aggregating (gelation) effect of calcium in regard to such protoplasmic properties as elasticity and membrane repair (page 444).

Certain workers have been inclined to group the elements to which protoplasm is permeable on the basis of valence and to predict that if one monovalent cation (sodium) affects protoplasm in one way, another monovalent cation (potassium or lithium) will do likewise. It is also assumed that bivalent ions (calcium, magnesium, barium) will affect protoplasm alike. There appears to be some justification for this in the behavior of nonliving systems (gelatin), but among living things the law does not hold (see also page 440). Gellhorn finds that sodium (monovalent) and magnesium (bivalent) both increase the permeability of sea-urchin eggs to dyestuffs, while calcium (bivalent) decreases it. Brooks and Gellhorn thus both find that monovalent sodium and bivalent magnesium have identical effects upon protoplasm; but Gellhorn finds that these metals *increase* the permeability of *animal* cells, while Brooks finds that they *decrease* the permeability of *plant* cells. Again do experimental facts indicate the danger of drawing all-inclusive deductions; and, after all, why should an animal egg react to a salt in the same way as a plant cell reacts to it? The reactions of the two *may* be the same, for cells wherever found have certain characteristics in common, but they *may* also differ as widely as do the plants and animals of which they are a part.

Another interesting problem is the accumulation of substances within the cell at a concentration greatly in excess of that in the surrounding solution. Here, again, we may be dealing simply

with a problem in metabolism and not with permeability. Plant nutrition is a question not only of getting certain salts and not others into the cell but of accumulating them. The law of diffusion states that substances pass in excess from a region of higher activity to one of lower activity, concentration being a factor. If we view the problem of accumulation as one of permeability, then the diffusion law is apparently not adhered to; but if we recognize that in all forms of metabolism one substance is used more than another, then accumulation is quite easily explained. The fresh-water alga *Nitella*, with cells up to 6 in. in length, lives in pond water where the amount of potassium is very slight, yet potassium may occur within the cell sap at a concentration one thousand times that of the potassium in the surrounding water. Hoagland and Davis first established these facts with accuracy. Osterhout and his coworkers continued the work on the large marine algae *Valonia* and *Halicystis*, both one-celled, bladder-like plants two or three centimeters in diameter, with a large central vacuole containing several cubic centimeters of sap. The contents of a number of cells will yield sufficient sap to permit an accurate chemical analysis. The results show that the concentration of potassium is over forty times as great within the *Valonia* cell as without, while sodium is only one-fifth and calcium one-seventh as concentrated within the cell as in the sea water; in other words, while there is forty times as much sodium as potassium in sea water, there is five and one-half times as much potassium as sodium within the cell. The total concentration of ions is approximately the same within and without the cell, only the proportions differ. Starfish, sea-urchin eggs, and many other cells contain a higher concentration of potassium than of sodium, though this relationship is reversed in the salt content of the sea water. Apparently, the cell has a mechanism by means of which it can hold more potassium than occurs in its surroundings and yet at the same time exclude sodium and calcium. If the problem is still considered as one in permeability, diffusion cannot be the explanation. Potassium may enter *Valonia* as much as two hundred times as rapidly as sodium, while, on the basis of ionic mobilities (the comparative rate at which ions move by diffusion), potassium should enter only six times as fast.

Now we come to the most extraordinary feature of the whole story. Growing in company with *Valonia* is its very closely

related cousin *Halicystis*. Analysis of the sap of this alga reveals that instead of there being five times as much potassium as sodium within the cell, as in the case of *Valonia*, there is eighty-seven times as much sodium as potassium.

Should such differences in ionic concentration remain constant, they could be used to distinguish species, especially those species which show little difference in their morphological characteristics. We should then have a classification of plants based on chemical or physiological distinctions similar to the protein classification of Mez (page 504) and Moyer (page 385).

A further step has been made in this direction by S. C. Brooks, who finds that *Valonia utricularis* growing at Naples shows little preference for potassium over sodium. *V. macrophysa*, on the other hand, shows a preference for potassium over sodium to the extent of 2.77 times. Species off Florida have a concentration of potassium five to twelve times that of sodium. *V. ventricosa* at Tonga has from four to ten times as much potassium as sodium. Brooks asks, Are we here dealing with physiological variants or with distinct valid species? Blinks has utilized these chemical distinctions in segregating part of the genus *Halicystis* as a new species.

A mechanism that permits two closely related plants growing in the same environment to show marked differences in their preference for sodium and potassium has been suggested by I. W. Bailey and C. Zirkle. It is assumed that acidity determines the rate of penetration of the two ions and that pH values within and without the cell are different. If potassium enters more rapidly on the alkaline side while sodium enters more rapidly on the acid side of neutrality, and if sea water is more alkaline than the cell sap (sea water has a pH of 8.2 and cell sap 5.8), then potassium will enter the cell easily; but once within, it cannot leave readily because of the acid condition of the cell contents. Sodium, on the other hand, will presumably enter slowly because of the unfavorable alkaline environment without but will leave readily because of the favorable acid environment within. The explanation is not based on permeability of the membrane; for if the membrane is permeable for potassium in one direction, it should be so in the other direction (if electrical forces are ignored). A difference in ionic activity is postulated on the assumption that potassium has a greater activity in an alkaline medium than in an acid

medium; this could be tested out apart from the plant. The explanation is speculative, but it rests on experiments with 30 dyes, in all of which the sodium ion entered rapidly at a pH of 3, less rapidly at pH 6, very slowly at pH 8, and not at all at pH 10; while calcium or potassium entered rapidly at pH 10, less so at pH 8, very slowly at pH 6, and not at all at pH 3. The hypothesis fits the case of *Valonia* well but fails when applied to both *Valonia* and *Halicystis* growing in the same waters.

F. C. Steward points out that certain other neglected factors materially affect permeability; thus, aeration of the aqueous medium containing the tissue with the carbon dioxide-free air causes increased salt absorption.

Theories of cell permeability rest mostly upon a postulate of membrane structure, under which subject they are best considered.

THE PROTOPLASMIC MEMBRANE

Introduction.—The surface layer of protoplasm, the so-called *plasma membrane*, is the first barrier that outside material must pass on entering the cell and the last that included material must pass on leaving. The membrane is, therefore, a very important part of the cell. The cellulose wall in plants is also an obstacle to diffusing substances but not so severe a one and not in the same sense as is the plasma membrane. Water, salts, and most organic substances pass through the cellulose wall freely, as through porous clay, while the plasma membrane has selective powers which are capable of change and adjustment. The cellulose wall may, however, play a more prominent part in permeability than heretofore suspected, but its role is probably always secondary to that of the membrane. There is considerable evidence, both direct and indirect, to support the presence of a plasma membrane around cells. If we recognize that the law of Willard Gibbs is, at least to a degree, applicable to protoplasm, then those substances which lower surface tension will become concentrated at the surface. Fatlike and other substances in protoplasm which thus affect surface tension will tend to collect at the surface and give to the outer layer a chemical constitution differing from that of the inner protoplasm. In addition to Gibbs' phase rule, there is other evidence that the surface of protoplasm is substantially different from the interior. All fluid surfaces differ from the

interior of the masses that they subtend, because they are subjected to different influences on their two sides. Conductivity measurements indicate that the surface of protoplasm differs from the interior. There is also anatomical evidence that a definite morphological membrane surrounds cells.

One important fact in regard to the plasma membrane must be borne in mind: It may possess an outer layer of fat which is essentially a nonliving film, but the membrane as a whole is of protoplasm, and while differing in constitution from the inner material, it is still living matter, as irritable and capable of change and adjustment as is protoplasm generally.

Physical Properties.—The protoplasmic membrane may be of any degree of substantialness, from that of a delicate, imperceptible film to a tough, anatomical pellicle. The former type of membrane is at the surface of most animal cells and of so-called "naked" masses of plant protoplasm such as the plasmodium of slime molds. The substantial type of membrane forms the covering of unicellular organisms such as the protozoan *Euplotes* (Fig. 28). No one doubts the presence of a pellicle on *Euplotes*. Less certain in the minds of some is the existence of a membrane on animal tissue cells and naked plant protoplasm. It has long been debated whether the red blood cell is a bit of firm jelly devoid of a covering, or a sac with hemoglobin in it. The presence of "ghost" cells in blood suggests the second alternative. The question has apparently been settled by microdissection, which has shown that blood cells possess a resistant and highly elastic membrane (Fig. 47). Whether delicate or substantial, and no matter on what kind of cell, the protoplasmic membrane is never permanently of the same quality. It is of living matter and therefore constantly undergoing changes—usually impereceptible but often very conspicuous ones. Amoeba and slime molds move about by the process known as amoeboid movement, which involves the formation of finger-like protrusions or pseudopodia. When these are formed, a change occurs in consistency from the firm, highly viscous state of the quiescent membrane to the fluid condition of the moving membrane. That such a change in the physical state of the surface layer of Amoeba occurs can be readily demonstrated by microdissection. The membrane when quiet offers considerable resistance to, and is torn by, a micro-needle; but when a pseudopodium is advancing, the surface layer

can be made to flow more rapidly by placing a needle in the pseudopodium just behind the advancing tip and then moving the needle forward. When the pseudopodium comes to a standstill, forward movement of the needle tears through the surface layer; the former fluid membrane has become a firm one. Pronounced changes take place in degree of delicacy, consistency, and tension of the plasma membrane coincident with physiological and structural changes in the cell. Division involves change in the protoplasmic surface. Especially evident is this in the case of the heavy pellicle of a protozoan, but equally true is it of all cells, in particular those which divide by the process of fission, or pinching in two.

Abnormal surface changes occur in cells. The sea-urchin egg is a quiet, spherical droplet of protoplasm. It may, on occasion, assume amoeboid activity when pseudopodia are put out from its surface, as in Amoeba. The normally smooth contour of the cup-shaped, red blood corpuscles may form surface protrusions, and the unripe sand-dollar egg may, in the presence of sperm, send forth extraordinary protoplasmic processes (Fig. 175). (These latter may be distortions of the surface layer or inner protoplasm oozing out through the membrane.)

Attempts have been made to determine the thickness of the protoplasmic membrane. Remington estimates 100 A. U. for the surface layer of the cell of the beet. Such figures apply to cells that do not change their form, as is true of those belonging to average plant tissue. The thickness of the membrane of an active amoeba changes greatly. Difficulty in determining not only the thickness but the actual presence of a protoplasmic membrane is increased by the fact that there is not usually a very sharp boundary at the inner surface of the membrane. The membrane often appears to grade imperceptibly into the inner protoplasm.

The Membranes on Cell Inclusions.—What is true of the surface of the cell is true of most cell inclusions. The presence of a nuclear membrane can be demonstrated in a convincing way after the nucleus coagulates, for the membrane is then capable of isolation as a stiff veil (Fig. 48). It is possible that this membrane is a post-mortem product, especially in the light of the behavior of some nuclei, which Chambers has shown may be severed while in the cell and the halves fuse on coming into con-

tact again, thus duplicating the behavior of liquid droplets. All available evidence indicates that the nuclear membrane is similar in quality and behavior to the outer protoplasmic one.

The membrane enclosing the vacuole of plant cells has been the subject of much investigation, because it is presumed by some to be the membrane upon which rest the osmotic properties of the cell. To this membrane deVries gave the special name *tonoplast*. Doubt has been cast on the reality of the vacuolar membrane. There are those who point out that if the inner layer of streaming protoplasm is carefully observed where it comes in contact with the vacuolar sap, particles will be seen moving in the vacuole carried along by the adjoining flowing protoplasm. The observation suggests that if the surface of the protoplasm, where it touches the vacuole, is flowing, as it must be to move particles in the vacuole, then there can be no true membrane. The weakness in this argument lies in the so often met with failure to appreciate that the protoplasmic membrane is itself living protoplasm and may

undergo the same changes in activity, in particular viscosity, as does the inner protoplasm which it bounds.

It is possible, with the aid of micro-needles, to withdraw an entire (plasmolyzed) protoplast (the cell contents as an intact

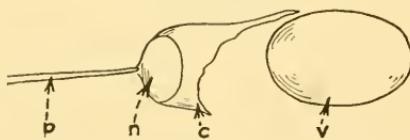


FIG. 142.—The vacuole (*v*) of a (now dead) plant cell separated by a needle (*p*) from the cytoplasm (*c*) and nucleus (*n*) and floating apart as a sack.

entity) from the cellulose enclosure of a plant cell (Fig. 142). Once so isolated, the protoplasm coagulates; it may then be stripped off from the vacuole, leaving the latter freely suspended, floating like a water-filled sac; this is only possible because the aqueous vacuolar sap is enclosed by a membrane. Any part of such a dismembered cell is, of course, not normal, but J. Plowe has isolated living intact cells in a similar way and found evidence from miscrodissection that the tonoplast—the vacuolar membrane—is a distinct morphological structure. That the protoplasm is still alive is indicated by a continuation of streaming. The Austrian plant physiologist Karl Höfler, in similar experiments, shows the tonoplast to be fluid, as indicated by streaming, but to possess, though fluid, considerable resistance and resilience, just as the liquid membrane of a soap bubble is resistant to pressure. Höfler also regards the tonoplast as functioning some-

what differently from the outer protoplasmic membrane. He believes the tonoplast to be less permeable to certain substances than the outer membrane and more permeable to water.

Permeability studies, micromanipulative work, and electric potential measurements all indicate that the inner protoplasmic (vacuolar) membrane is a distinct entity differing from the inner protoplasm and possibly functioning otherwise than the outer membrane. Thus viewed, the plant cell, as a permeability system, consists of external solution/outer protoplasmic membrane/protoplasm/inner protoplasmic (vacuolar) membrane/cell sap.

The cell membrane is formed of material that comes from the protoplasm. This is particularly evident when the membrane is repaired after being torn by a micro-needle. The newly exposed surface is formed by readjustment of inner material. That there is a readjustment of material, *i.e.*, that the physico-chemical and therefore permeable properties of the membrane differ from those of the inner protoplasm, is shown by the work of M. H. Jacobs. He demonstrated that a cell stained with dyes (neutral red) gives the color of an acid condition when ammonium chloride is *injected* into the cell but when the cell is *bathed* in this salt, it loses its color because only ammonia enters through the membrane, and hydrogen chloride remains behind. When acid sodium carbonate is injected, an alkaline reaction within the cell results; but when the cell is bathed in this salt, it gives an acid reaction because of entrance of carbon dioxide alone.

The following conclusions may be drawn: There is a demonstrable protoplasmic membrane. It is made of matter coming from the inner protoplasm but differs from it in total make-up. The membrane is capable of pronounced changes in its physical properties. It is often optically indistinguishable from the inner protoplasm of which it is an intimate part. It is reformed very rapidly when torn. It exhibits permeability qualities which differ from those of the inner protoplasm. It is rarely capable of being lifted off except in the coagulated state.

Chemical Constitution.—Many a theory or experiment is condemned and buffeted about by criticism only to emerge in the end as a more worthy contribution than any that followed it. Such is the experimentally supported theory of Overton, whose

suggestion of the mechanism of cell permeability and the protoplasmic membrane is still applicable. Overton centered his attention on fat solvents such as alcohol, ether, chloroform, xylene, methane, etc., and found that they all enter the living cell very rapidly. Salts, on the other hand, enter very slowly. He therefore believed that the plasma membrane is chiefly lipoid. Lipoid is a word freely used by biological chemists but not of very precise meaning. It includes those substances which are not true fats or oils but resemble them in being "oily" in nature (page 463). Lecithin and, less accurately so, cholesterin (or cholesterol) are among the best known lipoids. Lecithin is better classified as a phosphatide, and cholesterin as a sterol. Solvents (chloroform, etc.) of these fatlike substances quickly dissolve their way into the cell, while water-soluble salts enter very slowly. Overton neglected the salts, but his own hypothesis permits an interpretation of the entrance of salts into the cell on the basis of the hydrophilic nature of lipoids. Lecithin becomes milky when shaken in water; this means that it has taken up water and is permeated by it without going into true solution. After years of doubt and criticism, biologists have returned to a modified interpretation of Overton's hypothesis. It is now generally recognized that the protoplasmic membrane is fatty in nature at its outer surface but only like fat, for the true fats and oils, such as olive oil and petroleum (Nujol) oil, do not enter the cell at all.

It has long been known (since 1846, when first shown by DuBois Reymond) that the electric resistance of tissues is very high. This quality may rest upon the selective permeability of the cell membrane (for, if cations can pass and anions not, then no current can penetrate, and the resistance will be high), or the fatty nature of the membrane may be responsible. Osterhout takes this latter view and assumes that high electric resistance of the protoplasmic surface of the alga *Nitella* can be accounted for only if the membrane is of nondissociable, nonaqueous substances, *i.e.*, of fatty material.

The high protein content of protoplasm, which suggests the presence of protein at the surface, stands in contrast to the above evidence in support of a fatty nature of the cell membrane. Furthermore, the elastic properties of the membrane require it. Nathanson thought the cell membrane to be a mosaic of fat and protein.

The Sieve Hypothesis.—The foregoing considerations of the chemical constitution of the protoplasmic membrane give some indication of how it may function as a selectively permeable system. We may now consider this latter property of the membrane in greater detail.

There are three main hypotheses of the mechanism responsible for the selective properties of the protoplasmic membrane. One views the problem mechanically; the other, chemically; and the third, electrically. A membrane which functions in a purely mechanical way may be likened to a sieve with pores that allow fine particles but not larger ones to pass through. The chemical view of membrane mechanics postulates solubility as the determining factor. Substances enter the cell by dissolving their way through. From an electrical point of view, the membrane is regarded as charged and therefore permits only particles of one sign to pass.

The German physiologist Traube (1867) is primarily responsible for the suggestion that the plasma membrane functions as a molecular sieve. The membrane is presumed to have pores that permit small molecules but not large ones to pass. A membrane satisfying these conditions bears Traube's name. A Traube precipitation membrane is formed when copper sulphate comes into contact with potassium ferrocyanide (Fig. 101). A crystal of the former is put into a solution of the latter, and a membrane of copper ferrocyanide is precipitated around the sulphate crystal. This membrane permits the passage of water, but salt molecules are held back.

In so far as it exhibits a certain amount of selective permeability, the copper ferrocyanide membrane emulates the living plasma membrane, but let us see if the analogy is very close. This question has been answered by Collander. He showed that the behavior of the two is quite different, as the table¹ on page 282 indicates:

It is evident that there is no parallelism at all between the relative permeability of the living cell (*Rheo discolor*) and that of the artificial membrane. But the molecular volumes of the substances and their relative solubility in ether indicate two important facts—that the rate of entrance of the substances into the artificial membrane is a function of their size and that the rate of

¹ From R. Höber.

entrance into the living membrane is a function of their ether solubility. This means that the copper ferrocyanide membrane operates as a sieve and that the permeability of the plasma membrane is a question of relative solubility.

While the foregoing evidence is against the sieve hypothesis, there is other evidence in its favor.

Substance	Relative permeability of rheo discolor	Permeability of copper ferrocyanide	Molecular volume	Relative solubility in ether
Methyl alcohol.....	125	+++++	8.2	0.273
Ethyl alcohol.....	71	++++	12.8	1.86
Valeramide.....	69	++	28.7	0.170
Ethyl urethane.....	59	0.637
Ethylene glycol.....	4.4	+++	14.4	0.0068
Diethylurea.....	2.0	0.0185
Glycerol.....	1.3	+++	20.6	0.0012
Methylurea.....	1.2	0.0012
Urea.....	1.1	++++	13.7	0.0005
Glucose.....	1.02	+	37.5	0.0001
Glyecoll.....	1.0	+++	17.1	0.0001
Saccharose.....	1.0	+	70.4	0.0001

M. H. Jacobs concludes that the pore theory is the only one that can be seriously considered as offering an adequate explanation of the behavior of the erythrocyte in the presence of ions. S. C. Brooks regards the cell membrane as a sieve with pores the diameters of which change with surrounding conditions. The sieve, therefore, is not a fixed one but is capable of constant change and adjustment. A change in the size of the pores of a living or a nonliving membrane could be brought about by aggregation (coagulation), peptization (dispersal), change in sign and magnitude of electric charge, and the orientation of surface molecules. When small particles aggregate into larger ones, the spaces between the particles will become larger; the sieve, therefore, coarser. The reverse change takes place on peptization. A positively charged membrane would repel positive particles and thus keep them out; a negatively charged membrane would permit them to enter (no other factors interfering). If polar molecules are oriented at the surface, they will form a molecular sieve. We may compare the surface molecules to logs

weighted at one end and floating in a lake. The weighted end is down, owing to greater attraction at that end (due to gravity in the case of the logs; solubility or like factor, in the case of the molecules). Such logs or molecules in vertical formation and therefore closely crowded, or in oblique and entangled formation and therefore separated, will form a surface layer more or less permeable to other bodies, depending on the size of the latter (see further, page 289).

Estimations of the size of colloidal pores have been made for a number of nonliving substances. Bjerrum gives 1 to 90 $\mu\mu$ as the size of colloidal membrane pores. The smallest silica gel pores are 5 $\mu\mu$.

The sieve hypothesis has its weaknesses, but it has often been criticized on unjustifiable grounds. Thus, the behavior of the two forms of *d*-galactose and other sugar-permeability phenomena already referred to cannot be explained in terms of size of the sugar molecules. The same is true of glycerol and monoacetin, the molecules of which are of very nearly the same size, yet they enter the cell (as judged by hemolysis of red blood corpuscles) at a rate nearly fifteen times as fast in the one case as in the other (220 sec. for glycerol and 15 sec. for monoacetin). The first of these is very poorly soluble in lipoid material, and the second a good lipoid solvent; solubility, therefore, rather than size of molecule, is the more likely determining factor. But actually, as in the case of most if not all sugar-permeability phenomena, the question is not one of permeability primarily but of metabolism. The cell is freely permeable to both forms of the sugars, but it uses one in metabolic processes and not the other. The greater "permeability" of the one is therefore a matter of relative concentration within and without the cell.

In an attempt to make the sieve hypothesis fit certain selective properties of the protoplasmic membrane, the following speculation has been indulged in. The different rate of penetration of substances of the same molecular dimensions is explained by imagining that the cell membrane is a sieve with pores just large enough to permit the substances, say ions, to enter. Once inside, the ion is supposedly converted from the ionic condition, in which it entered, into a molecule, or from a smaller molecule into a larger one, and so cannot now leave through the sieve; other ions are not so changed and may therefore pass out again. There is

another possibility. One kind of ion, once within, may be held by adsorption to an organic molecule, while the other is not so held; the latter being free, could diffuse out again. Still another suggestion is that the membrane is electrically charged; a substance could enter as an electrically neutral molecule and, once within the cell, become ionized; it could then not leave, because as an ion it is repelled by the membrane. Proof that such changes take place are to be had from work by M. H. Jacobs, who found that ammonia can enter the cell only as a molecule, in an alkaline state, and that, once within, it dissociates and cannot leave.

The Solution Hypothesis.—Substances that dissolve fats should readily pass through a membrane made of fat. Thus thought Overton, but the membrane that he postulated was of lipoids and not true fats, and all the fat solvents with which he experimented (alcohol, ether, chloroform, etc.) are also water soluble. They would, therefore, also enter were the membrane an aqueous one but perhaps not so rapidly as when passing through a layer of fat. In either case, the entrance would be by solution. It should be pointed out that Overton qualified his hypothesis by the condition that only such fat solvents can enter as are soluble both in lipid and in water—a fact often neglected. (As already stated, fat solvents that are not soluble in water, such as olive oil and Nujol (petroleum) oil, do not enter living cells at all.)

The American physiologist J. H. Northrop has made artificial collodion membranes and found that a membrane containing thiosulphate and immersed in a solution of iodine concentrates the iodine within the sac; a membrane containing sodium chromate and immersed in mercuric chloride concentrates the chloride ion; and a membrane containing calcium carbonate and immersed in acetic acid concentrates acetate ions. The permeable properties of these artificial membranes closely parallel those of the living membrane (see also page 45).

The making of models that exhibit certain permeable qualities much resembling those of the living membrane has been indulged in by a number of workers, notably R. Höber and D. T. MacDougal.

The Electrical Hypothesis.—Membranes surrounded by salts are electrically charged. Forty years ago, Wilhelm Ostwald suggested that this and other electromotive phenomena in tissues might arise because living membranes prevent or retard the

passage of ions of one electric sign while permitting ions of the opposite sign to pass. As a result of this selective behavior, electric stresses arise due to an ionic concentration gradient across the membrane. We may, with Ostwald, ascribe the electric forces at membrane surfaces to selective permeability, or reverse matters and explain selective permeability in terms of the electric forces. This is not arguing in a circle to the extent that it may seem. Proteins possess a feeble residual charge of their own even when quite free from electrolytes. Pauli found blood serum to be weakly negative after seven weeks of dialysis. The charge still remaining is due to ionization of the protein (page 485). This weak initial charge of a protein membrane is greatly augmented by the presence of salts.

Two directly opposed hypotheses of the electric behavior of membranes are equally plausible. They have been advanced by L. Michaelis, W. R. Amberson, and others. The two hypotheses are like the original one of Ostwald in that they ascribe the entrance of ions of one sign and the holding back of those of the opposite sign to a charge on the membrane. But which sign must the membrane be to prevent this or that ion from passing? If it is positive, it may repel cations and permit anions to pass or attract and hold anions and permit cations to pass. It cannot, therefore, in theory be said whether repulsion or attraction (adsorption) determines the permeability of a charged membrane for ions; only actual experimentation with membranes of known sign will determine how a membrane functions electrically. There is also the possibility of there being, as Ostwald said, a potential gradient across the membrane, which would mean that one side is negative and the other positive.

Amberson, in observing reversal in the selective permeability of membranes, was able to answer the question as to the way in which a charged membrane may function. When the living skin of the frog is removed from the body and bathed in Ringer's solution, a difference of potential is produced across it, the inner surface being electropositive. The frog skin probably consists in large measure of protein; and as proteins are amphoteric, they, and therefore the skin, will be positive when bathed in acid and negative when bathed in alkali. The point at which this change takes place is the isoelectric point and is expressed in terms of acidity. The frog skin is negative when above (on the alkaline

side of) its isoelectric point. In this condition, cations penetrate the membrane more readily than do anions. In other words, the alkaline membrane retards the movement of those ions which are of the same electric sign as itself and permits the movement of ions of the opposite side. On the acid side of the isoelectric point, conditions are reversed. The membrane is now positively charged, and anions pass through more readily than do cations.

Mond carried out a similar experiment with red blood cells. Assuming that the membranes of erythrocytes are electropositive, he succeeded in changing their natural anion permeability into cation permeability by changing the sign of the membrane. By adding hydroxyl ions until the pH of the surrounding solution is above 8, the previous permeability of the membrane for chlorine and bicarbonate anions was changed to permeability for the cation potassium (through exchange with the external sodium ions). As the isoelectric point of globine is also about 8 (pH 8.1), this amphoteric protein is assumed by Mond to be the permeability-controlling substance in the membrane of red blood cells. The deduction is in keeping with certain (protein) theories of protoplasmic and membrane structure.

Permeability studies have, for the most part, been concerned with the passage of the ions and molecules of dissolved substances; less attention has been paid to the passage of water through membranes. The extraordinary fact has been mentioned that certain membranes appear to be more readily permeable to water in one direction than in another. Such behavior can be explained on the basis of electroendosmosis. Since the time of Helmholtz, chemists and biologists have followed him in interpreting the passage of water through membranes in terms of charge involving a typical Helmholtz double layer (Fig. 163), which leaves the membrane negative or positive. Through such a negative membrane water travels to the cathode; when the membrane is positive, the water travels to the anode. In the discussion on electroendosmosis, we considered only the one case of a glass capillary which selectively adsorbs the negative (OH^-) ion of water, leaving the positive (H^+) ion free to move. Other types of capillaries, *e.g.*, those of proteins, such as the pores in a gelatin membrane, may adsorb the other, positive ion and leave the negative ion of the water free to move; in this case, the water would migrate to the anode. Which ion a

substance will adsorb can be ascertained only by experimentation.

A similar speculation based on electrostatics has been advanced by O. Raber. The membrane is viewed not as a charged porous mass but as a colloidal suspension the elements or particles of which are charged and react with ions in the same way as do the particles of a colloidal suspension when a salt is added.

Surface Tension.—Czapek suggested that a substance must lower the surface tension of protoplasm in order to enter a cell. A high surface tension would present an interfacial membrane made up of tightly packed molecules between which substances could not pass readily. Low surface tension would mean a loose arrangement of surface molecules and therefore a more permeable membrane. In order to lower the surface tension of protoplasm, a substance must have a tension value less than that of the protoplasm. The hypothesis met with drastic criticism to which Czapek made the reply that it must collapse only when one substance has been found which, in spite of a surface-tension value below that of protoplasm, does not enter.

Adsorption.—Otto Warburg discarded all hypotheses of selective permeability for one of adsorption. He found that the degree of toxicity of the methyl, ethyl, propyl series is much more closely related to the adsorptive powers of these alcohols than to their surface tension or lipoid solubility. Those alcohols and other substances that are more strongly adsorbed to the surface get in the more readily.

The Emulsion Hypothesis.—Clowes found a striking analogy between the behavior of emulsions and the experimental permeability data of Osterhout. As a result, he developed an ingenious hypothesis of membrane permeability based on the behavior of emulsions. Osterhout had found that sodium chloride increases and calcium chloride (sometimes) decreases the permeability of cells. Loeb had previously found an "antagonism" between sodium and calcium, that is to say, the two elements when present in proper proportions prevent the toxic effect of each other. This proportion is that which exists in physiologically balanced solutions (sea water and blood). Clowes found that sodium (the hydroxide) causes the formation of emulsions of the oil-in-water type, while calcium (the chloride) brings about reversal to the water-in-oil type. Sodium and

calcium, in the proportion of 100 molecules of the former to 1 or 2 of the latter, which is approximately the proportion in which these elements occur in sea water and in blood, balance one another. The mixture has no effect on emulsions. Clowes viewed the plasma membrane as a fine emulsion; when oil is dispersed in water, the membrane is more permeable (to water-soluble substances, *i.e.*, salts); when water is dispersed in oil, the membrane is less permeable. The former condition is presumably produced by sodium, and the latter by calcium. Normally, the protoplasmic emulsion is in a state of equilibrium near the reversal point, for it is bathed in a balanced solution. It is, therefore, readily thrown one way or the other by a change in concentration of the salts in the surrounding medium (Fig. 83).

The best support of Clowe's hypothesis is the fact that most oil emulsions are at the reversal point when sodium and calcium are present in the same proportion as they occur in physiologically balanced solutions. The hypothesis met with adverse criticism on the grounds that there is no evidence that the protoplasmic membrane is a fine emulsion, and it is the hydroxyl (OH^-) anion rather than the sodium cation which reverses and holds emulsions in the oil-in-water state. In reply to these criticisms, the English botanists Dixon and Clark said that an electrical stimulus affects emulsions in the same way as it does protoplasm. An electric current will cause an emulsion, originally almost impermeable to ions and water-soluble substances, to become fairly permeable, which is the same effect that electric stimuli have on living tissues; *viz.*, they increase permeability. Dixon and Clark, therefore, conclude that a hypothesis of the structure of the plasmatic membrane that explains two such apparently unconnected and remarkable phenomena as the antagonistic action of certain ions on permeability (the hypothesis of Clowes) and the permeability changes produced by electric stimuli (the work of Dixon and Clark) deserves serious consideration. This is true, yet it may simply mean that two rather diverse types of systems (an emulsion and a living jelly) show similar responses to the same environmental changes.

We are forced to discard the emulsion hypothesis of membrane control in spite of two substantial facts in its support (that of Clowes and that of Dixon just cited) because of the following reasons: There is no direct evidence whatever of phase reversal

in the protoplasmic emulsion. It is very unlikely that protoplasm could exist as a living substance if fat were the continuous phase. (Metabolic reactions take place in an aqueous medium.) As the stability of an emulsion increases with decrease in size of the dispersed particles, owing to a great increase in the surface tension of the stabilizing membrane (see page 128), the ultramicroscopic emulsion (if it exists) would be extremely difficult to reverse. And, finally, the amount of fat in the dispersed globules of an ultramicroscopic emulsion is probably insufficient to enclose the aqueous medium.

Role of Fats.—The permeability problem is intimately associated with the role of fats in the life of the cell. It is generally agreed that the outer surface of the plasma membrane is coated with fatty material. This being true, we must grant the possibility of its being emulsified, but that permeability control is determined by the behavior of an emulsion is, we have seen, very unlikely. There is another interpretation of the role of fats at the surface of cells which in many respects is more in keeping with the present-day theories of the behavior of oils at interfaces. This interpretation is in continuation of the idea already expressed, that polar surface-molecules are comparable to logs floating in a more or less upright position, thus forming a sievelike structure. If these imaginary logs are fat molecules, then their position will be determined, in large measure, by their polarity. The polarity of fats, in the technical chemical sense, is not high, but their molecules are linear, and they do form oriented mono- or bimolecular films. The molecule of the fatty palmitic acid is (after N. K. Adams) 24 A. U. in length and 4.7 A. U. in diameter (21 sq. A. U. in cross section), five times as long as the mean diameter. Tripalmitin occupies about the same area as three molecules of palmitic acid (*i.e.*, 63 sq. A. U., or about 8 A. U.²). One dimension of the tripalmitin molecule is therefore three times the mean of the other two. Lecithin does not appear to give a compact "condensed" film. The area presumably occupied by the molecule is consequently greater (13 A. U.²). The long dimension is, however, at least twice the mean of the other two in an "expanded" film. That these linear fatty molecules are polar and therefore orient must be granted from the fact that they form monomolecular films on water, because one end of the molecule has an affinity for water, and the other not.

In all probability, the films formed are usually two or more layers thick and so arranged that the oily ends are in contact with each other, and the acid ends (the glyceryl-cholyl-phosphoric acid in the case of lecithin) are in contact with the water or with each other if the film is polymolecular. The films of soap bubbles are probably thus arranged. The iridescence of soap bubbles is due to the interference of light by the lamellae of the polymolecular film, and the kaleidoscopic change in color is due to the sliding of the soap leaflets one upon the other where they are in contact along their oily surfaces. It is the orientation of such linear and polar molecules that best accounts for the part that fatty substances play in determining the permeability qualities of the plasma membrane.

The Donnan Equilibrium.—We return again to the Donnan equilibrium (page 203) in explanation of a biological phenomenon, *viz.*, selective permeability in terms of the unequal distribution of ions on the two sides of a membrane. Donnan has himself shown how membrane equilibrium can be applied to permeability problems. Molecules and ions within and without a red blood cell may be distributed as follows:

Red Blood Cell	Blood Serum	
Hb	P	Hb = hemoglobin molecule
Hb ⁻	P ⁻	Hb ⁻ = hemoglobin anion
Cl ⁻	Cl ⁻	P = serum protein molecule
HCO ₃ ⁻	HCO ₃ ⁻	P ⁻ = serum protein anion
Na ⁺	Na ⁺	
K ⁺	K ⁺	

If these two solutions are very dilute, it is possible mathematically to determine their distribution (concentration) on the two sides of the membrane (within and without the blood cell). This is true only if there are no other interfering factors, as is so often the case in biological systems when an attempt is made to interpret them in terms of relatively simple physicochemical systems.

Narcosis.—The first definite hypothesis of narcosis was that of Overton (page 280), who stated that the narcotic effectiveness of a substance is proportional to its lipid solubility. If the cell surface is coated with lipoids, substances that dissolve them will enter very rapidly. The theory is essentially one of disturbance in cell permeability. It is true that narcotics dissolve lipoids and that they enter the cell very rapidly; a lipid-permea-

bility theory of narcosis may, therefore, be justly formulated, but the theory fails when applied to other forms of anesthesia, *e.g.*, when caused by salts. Magnesium sulphate is an excellent narcotic; chloral hydrate is a still more powerful one, and it is several times more soluble in water than in oil. While the parallelism between lipoid solubility (disturbance of permeability) and narcotic action is not a perfect one, yet high lipoid solubility is typically associated with pronounced narcotic action.

Permeability of the Protoplast as a Whole.—Permeability studies are usually based on the assumption that there is a plasma membrane and that it is responsible for permeability phenomena. As the membrane is of protoplasm, there is no reason why the permeability properties assigned to the membrane should not be applicable to the protoplasmic mass as a whole. Indeed, some workers ascribe all permeability phenomena to the protoplasm as a whole and not to the surface layers alone. Protoplasm is a jelly; the taking up of water by it must therefore involve imbibition, and this is but a form of permeability. Freundlich emphasizes the importance of swelling in natural processes. Höfler states that the permeability of protoplasm for water is not unlimited but, on the contrary, is rather slight; *i.e.*, there is control (see also page 278). De Haan says that changes in the water permeability of protoplasm are the expressions of changes in the swelling of protoplasm. In the same way that the water permeability of the cell is determined by the entire protoplasmic mass, just so may certain permeability phenomena be properties of the protoplasm as a whole instead of properties of the surface layer alone.

Conclusion.—All of the foregoing hypotheses of membrane mechanics have an element of truth in them. Pore size, chemical constitution, electric charge, surface tension, adsorption, imbibition, the orientation of linear molecules, and the Donnan equilibrium, operating collectively rather than singly, probably determine the selective permeability of the living protoplasmic membrane.

CHAPTER XVII

ACIDITY

No other quality of protoplasm and of the fluids that bathe it has received more attention of late than has acidity. Perhaps undue emphasis has been laid upon acidity; nevertheless, it is still recognized as one of the most important conditions upon which the prolonged life and continued well-being of organisms rest. Body fluids are maintained fairly constantly at a definite acid or alkaline value; pronounced changes usually result in an unhealthy condition. The acidity of the surrounding solution (of the soil water, the pond water, or the ocean) is quite as important in the case of plants and aquatic animals as is that of the body fluids. Many species of plants are so sensitive to the acid condition of the soil that if the degree of acidity is not suitable, the plant will not survive. Other species, however, show great tolerance. The nonliving world is also very sensitive to its acid condition. The rate and type of chemical reactions are in large measure dependent upon acidity.

Terms.—An *acid* is a compound that yields hydrogen ions when in solution, or, an acid is a compound the hydrogen of which is replaceable. Alkalinity is the opposite of acidity. An *alkali* is a substance containing the hydroxyl radical and is known as a *base*. The amount of replaceable hydrogen in an acid can be ascertained by the amount of alkali necessary to neutralize the acid.

Degree of acidity may be determined and expressed in a number of ways. The taste of a substance indicates whether it is very sour, mildly sour, or not sour at all. Such a method serves well for a crude determination of the acidity of food, but it is obviously not precise, though many a chemist has used his tongue as an acid indicator. Some dyes assume different colors when in solutions of different degrees of acidity; the color is usually definite for a definite degree of acidity. Acidity may, therefore, be determined by color. This is done by comparison

with a solution of known acidity. The method is much used, and the indicator dyes have been very carefully calibrated. In view of the fact that certain ions are responsible for acidity, then any solution having these ions should have a measurable potential. This is true, and the electrical method is a very accurate one for determining acidity when conditions are favorable.

Hydrogen is the element responsible for acidity. All acids contain it. An acid may be defined as a substance which in solution yields hydrogen ions. The older classical definition of an acid terms it a compound containing hydrogen which is replaceable by a metal. While the first definition is the one now generally accepted, certain features of the older viewpoint still persist, such as the custom of expressing concentrations of solutions in terms of *normality*. The two definitions differ in that one lays emphasis on the activity of hydrogen *ions*, while the other includes *all* hydrogen replaceable by the metal of a base, whether the hydrogen is ionized or not. Sometimes one or the other is referred to as the *true* acidity, but in doing this we simply lay emphasis on that physical, chemical, or physiological property in which we are primarily interested. Both viewpoints are correct. The chemist thinks of acidity in terms of all the replaceable hydrogen (normality) because this method is valuable to him. The physiologist centers his interest on the ionized hydrogen because it is the hydrogen ions which determine acidity for the organism. Let us take up the older viewpoint of acidity first by considering what is meant by normality.

Normality.—Hydrogen makes for acidity; the hydroxyl group (OH) makes for alkalinity; the two neutralize each other. When an acid is mixed with a base in proper proportions and in solution, a neutral salt and water result ($\text{HCl} + \text{NaOH} = \text{NaCl} + \text{H}_2\text{O}$). A molecule of an acid containing one atom of replaceable hydrogen will just neutralize one molecule of a base, that is to say, combine with it so as to produce a salt. This relationship is known as *normality*. A *normal* solution of acid is one containing 1 (the molecular weight) gram of hydrogen per liter replaceable by the metal of a base. A normal solution of hydrochloric acid contains 1 gram of hydrogen and 35.5 (the molecular weight) grams of chlorine in a liter, the molecular weight of hydrochloric acid being 36.5. A normal solution of sulphuric acid, H_2SO_4 , must also contain 1 gram of hydrogen in a liter. The molecular

weight of the acid is 98; of this amount, two are hydrogen; therefore one-half of the molecular weight, or 49 grams, in a liter will give 1 gram of hydrogen per liter. Acetic acid has four hydrogen atoms in its molecule (CH_3COOH), but only one of these—the H of the carboxyl group (COOH)—can be replaced by the metal (Na) of a base (NaOH). Consequently, to make up a normal solution of acetic acid, the whole molecular weight in grams (60) must be taken, the hydrogen atoms in the CH_3 group being ignored because they are not replaceable by the metal of a base. We must, therefore, when making up a normal solution consider whether the acid is monobasic, like hydrochloric, or dibasic, like sulphuric; we must also consider the total molecular weight, including any water of crystallization; and finally consider what part of the hydrogen is replaceable by a base.

A normal solution of a base is like that of an acid, except that we think in terms of the hydroxyl (OH) group instead of the hydrogen atom.

Acidity of the normality type, which we have been considering, is capable of measurement by *titration*; that is to say, the quantity of replaceable hydrogen can be determined by measuring the quantity of alkali that is exactly necessary to neutralize the solution. A color indicator (dye) may be used to determine the exact point of equilibrium between the acid and the base. Thus, if a dye such as litmus or phenolphthalein is added to an alkaline solution, the color is blue in the case of litmus and red in phenolphthalein. If, now, acid is added drop by drop, the amount being measured until the solution just turns in color (from blue to red in litmus and from red to colorless in phenolphthalein), then the solution is at (or near) the point of neutrality, and the amount of acid added just balances the alkali present.

Molarity.—The convenience of expressing concentrations in one way or another depends on our interest. If we are thinking of a solution as a physical system, capable of exerting, for example, osmotic pressure, then the total number of molecules (or ions) present is important. In this case, a concentration expressed in terms of total molecules is more helpful than one in which the concentration of hydrogen only is given. The physical chemist, therefore, uses normality less as a means of expressing concentration and *molarity* more. A *molar* solution

is one containing the molecular weight of the substance in grams per liter. A molar solution of hydrochloric acid contains 36.5 grams (the molecular weight in grams) per liter. This concentration has 1 gram of hydrogen in a liter; it is, therefore, also a normal solution. A molar solution of sulphuric acid contains 98 grams (the molecular weight in grams) of the acid in a liter. This concentration has 2 grams of hydrogen per liter; it is, therefore, twice normal.

Molar concentrations of all compounds contain the same number of molecules.

It is still necessary to express concentration in percentage when the exact constitution and therefore the molecular weight of a substance are not known, as is true in the case of many proteins.

Hydrogen-ion Concentration.—Let us now consider the second kind of acidity—that due to *free* hydrogen ions and not to replaceable hydrogen atoms. Acid qualities have been ascribed to one thing after another as science has advanced. Paracelsus imagined the presence of an *acidium primogenium*. Lavoisier thought oxygen to be the cause. And now hydrogen is known to be responsible for acidity, either hydrogen so placed as to be replaceable by a metal or hydrogen ions. From this latter viewpoint, only substances capable of supplying hydrogen bearing a positive electrical charge (H^+ ions) are acids.

Hydrochloric and acetic acids have certain properties in common but to a different degree when viewed in the light of their influence on physical, chemical, and physiological reactions. Physically, hydrochloric acid conducts electricity better than does a like (normal) concentration of acetic acid. Chemically, hydrochloric acid is a much more efficient catalyst in the breaking down of sucrose into glucose and levulose than is a like concentration of acetic acid. Physiologically, a normal solution of hydrochloric acid is a poison, while normal acetic acid is a weak artificial vinegar. Hydrochloric acid is, therefore, a stronger acid than acetic. These acid qualities rest on the proportion of free hydrogen ions present in the two cases. Only the hydrogen atoms of the carboxyl groups ($COOH$) of acetic acid molecules ($CH_3\cdot COOH$)—and of these, relatively few—dissociate; therefore, in a solution of acetic acid, there are mostly $CH_3\cdot COOH$ molecules and few $CH_3\cdot COO^-$ and H^+ ions (the percentage of

dissociation in a normal solution of acetic acid is but 0.04). When hydrochloric acid, on the other hand, dissociates, it does so fully (so we now believe) into H^+ and Cl^- ions; consequently, all of its hydrogen contributes to the ionic acidity (and therefore to the electrical conductivity, catalytic power, and poisonous effect) of the solution. In other words, in hydrochloric acid, all of the hydrogen is available as H^+ ions, which make for acidity; while in a solution of acetic acid, there are three kinds of hydrogen present—the bound hydrogen of the CH_3 radical, which contributes nothing to the acid properties of the solution; the hydrogen of undissociated $COOH$ radicals, which is available for exchange with the metal of a base and thus contributes to the titratable acidity, or normality, of the solution; and the free hydrogen ions from the dissociated carboxyl radicals, which alone represent the *hydrogen-ion concentration*, *i.e.*, the ionic, catalytic, and physiological acidity.

Living organisms are very sensitive to hydrogen ions. The biologist is, therefore, interested in ionic acidity rather than normality. The stimulus resulting from this interest has led to a great advance in knowledge of the properties of acidity in terms of hydrogen ions. This newer knowledge has come primarily from the Dane S. P. L. Sørensen, whose work in hydrogen-ion concentration was the first and most outstanding. Important also are the contributions made in Germany by Leonor Michaelis and in America by W. Mansfield Clark.

Dissociation.—The Dutch physical chemist van't Hoff became, as a result of his long and intimate friendship with his fellow countryman, the botanist Hugo de Vries, interested in the osmotic properties of solutions. This interest led to the discovery that the pressure of a substance in solution is the same as it would be if the substance were in a gaseous state occupying the same volume as does the solution. Thus do the gas laws of Boyle, Gay-Lussac, and Avogadro hold true for (dilute) solutions. Van't Hoff found, however, that when the dissolved substance is an electrolyte, *e.g.*, a salt, the gas laws do not hold strictly. Such solutions are osmotically more active than they should be; that is to say, they exert more pressure than does the same concentration of a nonelectrolyte (*e.g.*, sugar), though both contain the same number of molecules. Van't Hoff added a factor i to his formula for the behavior of solutions to account for

the proportion between the excess number of osmotically active particles and the total number of molecules known to be present in the solution. This factor i took care of the divergence of electrolytic solutions from the perfect gas laws, but it told nothing about the cause of the divergence.

The young Swedish chemist Arrhenius, who later was a student in van't Hoff's laboratory, interpreted the constant i of van't Hoff in another way. He assumed that salt molecules break down, or *dissociate*, when in solution, into their respective *ions*. (It is now believed that there is no breaking down of the salt molecules but merely a separation of the atoms or ions, because there are no molecules as such in the salt crystal.) But there is a slight discrepancy. The osmotic pressure of salt solutions is not quite proportional to the number of ions that should be present if dissociation is complete. The osmotic pressure of sodium chloride is not quite twice, and of calcium chloride not thrice, as great as is an equimolecular concentration of sugar. This fact led Arrhenius to make a further postulate, a corollary to his theory of dissociation. He proposed that some of the molecules of a salt do not break down into ions and that therefore the number of particles (molecules and ions) present in the solution is not quite twice (in a monovalent salt) that of the total number of original molecules. This is the theory of *incomplete dissociation*. As a result, we have so-called *dissociation constants* of electrolytes.

The dissociation constant K expresses the ratio between the product of the concentrations of the dissociated ions and the concentration of the undissociated molecules. It is a special case of the law of mass action and may be expressed as follows for a hypothetical electrolyte AB :

$$K_{AB} = \frac{[A^+] \times [B^-]}{[AB]}$$

Dissociation constants are not constant for strong electrolytes, nor do they indicate what it was originally thought that they indicated in strong electrolytes (as we shall see).

The dissociation constant K of picric acid is 1.4×10^{-1} (0.14), which is a moderately high value. The constant of the mild acetic acid is 1.86×10^{-5} (0.0000186), a low value; of the very weak boric acid, K is 6.4×10^{-10} . Expressed in percentage,

boric acid is 0.01 per cent, and acetic acid 0.4 per cent dissociated, while the strong nitric acid is 82 per cent dissociated. The salt, potassium chloride, is 86 per cent dissociated when at 0.1 molar concentration, which means that at a concentration of 0.1 *M* (about 0.7 per cent) 86 out of every 100 molecules of potassium chloride are dissociated into their two ions K^+ and Cl^- , while 14 molecules remain intact. The degree of dissociation, if the theory is correct, is dependent upon concentration. At dilute concentration, salts are more highly ionized than when concentrated. Infinite dilution yields complete dissociation.

Arrhenius advanced the dissociation theory of electrolytes in 1883. Twenty-one years later, the American physical chemist A. A. Noyes suggested a theory of *complete* dissociation. Noyes was interested in the optical activity and color of electrolytic solutions which are independent of concentration and therefore of the degree of dissociation. This led him to the conclusion that the salts studied were completely ionized up to a concentration of *M*/30 (a 0.2 per cent solution if the salt is sodium chloride). If the dissociation is complete, then why the anomalous osmotic behavior; that is, why, if there are two ions for every molecule, is not the osmotic pressure twice as great? Similar anomalous behavior exists in the electrical conductivity of solutions. Salts in solution conduct less current than they should if every molecule is dissociated into two ions. In postulating complete dissociation, Noyes must in some way explain the anomalous behavior, in osmotic pressure, electrical conductivity, freezing point, etc. He did it in terms of migration velocity—"the decrease in conductivity is due merely to a change in migration velocity." What this means we shall see in a moment. G. N. Lewis went further by saying that the experimental facts suggest complete dissociation up to a concentration of normal or half normal (normal sodium chloride is nearly 6 per cent). Then came the work of the Dane Niels Bjerrum, who, with others, has practically convinced the chemical world that many salts, even in high concentration, are *fully* dissociated into their ions and that all strong electrolytes if not fully dissociated are more so than was formerly thought to be true. The contribution of Bjerrum is so important and so simply and clearly expressed in his original paper that we can do no better than to let him tell what happens in his own words.

I find that pure normal chromium salts (strong electrolytes) in solution always have exactly the same absorption of light (*i.e.*, the same color) no matter what the concentration. It is only in the case of the strong electrolytes that the color is not changed with the concentration. The color of the weaker electrolytes depends upon the concentration. I suppose that in the solutions of the weaker electrolytes there is always a greater or smaller quantity of undissociated salt and that this salt not only causes the changes in color but also reduces the electric conductivity. These color relations found in connection with electrolytic dissociation can best be explained by changing Arrhenius' hypothesis in the following manner. We suppose that the strong electrolytes always are completely separated into ions and that this is the reason why they always have the same color in all concentrations. If changes of color take place in solutions of an electrolyte, the ions have more or less entered into combination with each other—the dissociation is not complete.

If this hypothesis is correct, then the decrease in molecular conductivity and in molecular depression of the freezing point that accompanies the increase in concentration must be due to the action of the electric charges of the ions on each other. The molecular conductivity is diminished not because the number of ions is decreased but because the ions move more slowly. It seems to me there can be no doubt that the electrolytic friction must increase with the ion concentration, both because the positive and the negative ions will more frequently collide than the neutral molecules and also because the electric field around the ions, increasing with the concentration, will create about the ions a water mantle of increasing thickness.

The change in migration velocity to which Noyes referred is due, then, according to Bjerrum, to the *electrolytic friction* of the ions. They are thus prevented from exercising full freedom in motion, with the result that their kinetic energy is lowered, and their osmotic pressure and conductivity reduced. It is the electrolytic interference that prevents twice as many ions in a salt solution having twice the (osmotic, etc.) effect of an equimolecular concentration of sugar.

Anomalous behavior of electrolytes is greater at high than at low concentration. This is true because the higher the concentration the closer are the ions and the greater is the interionic electrical friction. In very dilute solutions, the ions are farther apart, their mutual electrostatic attractions are negligible, and the ions can exercise their full kinetic energy.

Much work has been done in the past on the assumption of incomplete dissociation of strong electrolytes. This work need not now be discarded, because, while dissociation constants apparently do not indicate degree of dissociation, as was originally thought, they do indicate the degree of interionic friction and therefore the extent to which the electrolyte will diverge from the theoretically perfect behavior of solutions. This dissociation constant of potassium chloride does not indicate that this salt is only 86 per cent ionized, but it does indicate that the osmotic pressure of the salt is only 86 per cent of the total which it theoretically should be.

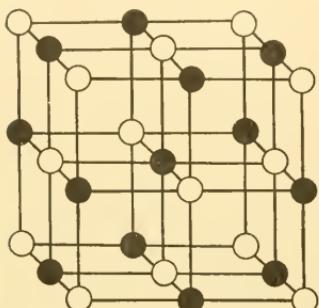


FIG. 143.—Space lattice of a sodium chloride crystal.

After a theory has been well established experimentally, it is always easy to see why it should be true and why we ought to have suspected it long before. The studies of William Bragg on crystal structure have shown that there are no molecules as such in crystalline matter and atoms are already ionized in a salt crystal (Fig. 143).

If, then, the atoms all exist as ions in the solid salt, why should they not remain so when in solution?

The complete dissociation theory of Bjerrum has been very generally accepted. Opposition, however, has existed, but it forces one only to grant the possibility that there may not be complete ionization in the case of some strong electrolytes. For weak electrolytes, the incomplete dissociation hypothesis of Arrhenius still holds in full, as these are always only partly dissociated. Electrolytes as a group, therefore, dissociate anywhere between the low value of water and the complete dissociation of strong electrolytes.

It must be borne in mind when considering dissociation that electrically charged particles of opposite sign, such as ions, in not too dilute aqueous solutions (and in nonaqueous solutions of even rather dilute concentration) may be held very close to each other owing to mutual attraction. This *ionic association* is electrical in nature. It is due to the forces that surround a charged body and is therefore not the same kind of bond that exists where compounds are formed by the sharing of an electron.

Such *paired ions*, held solely by electric attraction, are referred to as *ionic doublets*.

Other similar problems in connection with dissociation constantly come up, some of them rather disturbing in regard to accepted theories concerning the hydrogen ion. Lowry says that there is no such thing as a hydrogen ion in solution because there is no obvious reason why one and not both of the two hydrogen atoms of water should become detached. It is now a rather generally accepted hypothesis that the following reaction takes place when an acid is added to water:



A number of chemists, including J. N. Brönsted and M. Kilpatrick, have contributed to the newer concept of acidity. On the basis of their work, we no longer define an acid as a compound that can split off hydrogen ions but as a hydrogen compound that can split off protons (the hydrogen ion is a proton). In accepting this definition, we discard the requirement that an acid should combine with a metal hydroxide to form a salt and water and say merely:



From this point of view, water is an acid, for



so also are certain anions such as



and cations:



The newer concept of a base also discards the older definition, which requires that a base must give off hydroxyl ions, and defines it as a substance that can take up protons; water is thus a base.

The equation $\text{H}_2\text{O} + \text{HCl} \rightleftharpoons \text{H}_3\text{O}^+ + \text{Cl}^-$, which shows water to be a base, and the equation $\text{H}_2\text{O} \rightleftharpoons \text{OH}^- + \text{H}^+$, which shows water to be an acid, also indicate the amphoteric character (see page 483) of water.

In accepting these newer theories, we do not thereby altogether discard the older concept which ascribes acidity to the hydrogen ion.

The Dielectric Constant.—Any discussion of dissociation would not be complete without reference to the *dielectric* constant, which gives us a clue as to why electrolytes dissociate in water.

Pure water is a very weak electrolyte, *i.e.*, dissociates but little; pure liquid hydrochloric acid is an equally weak electrolyte; yet if these two substances are mixed, the solution is an excellent conductor of electricity, which means that water, though a poor conductor of electricity in itself, has the capacity to confer this property (dissociation) on another substance when the two are intimately associated. That property of water which is primarily responsible for the dissociation of hydrochloric acid in solution is termed the *dielectric constant*. The dielectric constant is that property of solvents which determines, to a great extent, their dissolving power and those other properties which they confer on substances dissolved in them, such as ionization.

The dielectric constant depends upon the nature of the medium and is determined by the electrical force or repulsion between two point charges separated by a given distance in the medium; in other words, the repulsive or attractive effect that two charged bodies have upon each other is dependent upon the electric character of the medium that separates them. The dielectric constant is a measure of that character, the unit being the dielectric constant of a vacuum. The value for water is 81; for ethyl alcohol, 26.8; and for acetic acid, 9.7.

Substances ionize in water because the mutual attraction of the ions of the dissolved substance is not sufficient to hold the ions together in opposition to the electrical barrier set up by the surrounding water. Water molecules are *polar*, *i.e.*, electrically unsymmetrical. The dielectric constant is the most direct evidence that we have of polarity in molecules.

Hydrogen Ions.—The formula for dissociation can be expressed again, thus:

$$K = \frac{c_1 \times c_2}{C}$$

where K is the constant; c_1 , the concentration of the cations (H^+ in acids); c_2 , the concentration of the anions; and C , the

concentration of the undissociated molecules (all expressed in moles or molecules per liter, *i.e.*, in equivalent concentrations).

For water, the formula becomes (brackets indicate concentrations)

$$K = \frac{[H^+] \times [OH^-]}{[HOH]}$$

therefore

$$K[HOH] = [H^+] \times [OH^-]$$

The dissociation of water is so slight that in the product $K[HOH]$, $[HOH]$ is considered constant and is expressed with K as K_w , the dissociation constant of water. The value of K for pure water is 10^{-14} (0.0000000000000001). As this, in the case of water, is the product of equal concentrations of $[H^+]$ and $[OH^-]$ ions, the concentration of each of these must be 10^{-7} .

The dissociation constant of acetic acid $[CH_3\cdot COOH]$ is

$$K = \frac{[H^+] \times [CH_3\cdot COO^-]}{[CH_3\cdot COOH]} = 0.000018$$

In a normal solution of acetic acid, about 43 molecules in 10,000 are dissociated (at 25°C.). As each dissociated molecule yields one hydrogen ion, the H^+ ion concentration is $43/10,000 N$. This is the numerical value of $[H^+]$ for acetic acid. The value of $[H^+]$ for a normal solution of the very weak boric acid is 0.0000255 N . This means that the actual weight of free hydrogen ions in a normal solution of acetic acid is 0.0043 gram; and in boric acid, 0.0000255 gram (in hydrochloric acid, it is 1 gram).

The concentration of hydrogen ions in any solution is obtained by transposing the general formula, thus:

$$c_1 = \frac{K \times C}{c_2}$$

For water, this becomes

$$H^+ = K \frac{[HOH]}{[OH^-]}$$

As the acidity of a solution decreases, the alkalinity increases, which means that with a decrease in hydrogen ions there is a corresponding increase in hydroxyl ions. Both reach a convenient limit in normal acid and alkaline solutions. These limits constitute the ends of the hydrogen-ion scale and are

expressed by the values 1 and 10^{-14} , which represent *the actual weight in grams of the hydrogen ions in a normal acid and a normal alkaline solution*. As the weight of H^+ ions in pure water is 10^{-7} gram per liter, while in a normal solution of alkali it is but 10^{-14} , some of the free hydrogen ions in water must be lost (taken up) when an alkali is added. The product $[H^+] \times [OH^-] = 10^{-14}$ remains constant in all aqueous solutions. Neither the selected maximum of the hydrogen-ion scale (1 gram of hydrogen per liter as represented by a normal solution of acid) nor the minimum (10^{-14} gram of hydrogen per liter as represented by a normal solution of alkali) is a true maximum or minimum, for twice or thrice normal acid and alkali possess a greater proportion of acid or alkaline ions. Normal hydrochloric acid contains 1 gram of hydrogen ions per liter; twice normal contains 2 grams; and thrice normal, 3 grams. Such concentrations are practically never met with in nature and rarely in physiological work. Consequently, normal acid and normal alkali give a convenient and practical range of acidity and alkalinity, from 1 gram to 10^{-14} gram by weight of hydrogen per liter. The value of 10^{-7} gram per liter of hydrogen ions for pure neutral water is the midway point and becomes an indication of neutrality (at 20°C.).

To express or plot as a curve the entire range in hydrogen-ion concentration from 1 to 10^{-14} gram, with 1 inch equivalent to the change from 0.0000001 to 0.000001 (10^{-7} to 10^{-6}), would require a sheet of paper 2,000,000 inches, or nearly 32 miles, long. Seldom is it necessary to plot so great a range, but often the range is extensive. It is evident that an abbreviated form in which to express hydrogen-ion concentration is needed. If we select any convenient mixture of, say, acetic acid and sodium acetate with a hydrogen-ion concentration of 0.000018 gram per liter, this value can be stated in any of the following ways:

$$0.000018 = \frac{18}{1,000,000} = \frac{1.8}{100,000} = \frac{1.8}{10^5} = 1.8 \times \frac{1}{10^5} = \\ 1.8 \times 10^{-5} = 10^{0.25} \times 10^{-5} = 10^{-4.75}$$

The simplest of these figures is the last or, with the 10 understood, just the negative logarithm, -4.75. The negative logarithm, therefore, is sufficient to express a hydrogen-ion value. The actual hydrogen-ion concentration, in grams per liter, of normal

hydrochloric acid is 1.0; of one-tenth normal acid, 0.1; of pure water 0.0000001; and of normal sodium hydroxide, 0.000000-00000001; these values expressed in terms of their logarithms become 0, -1, -7, and -14 (10^0 , 10^{-1} , 10^{-7} , 10^{-14}). In nature and in usual physiological work, the hydrogen-ion concentration is always less than one (that of a normal and fully dissociated acid); consequently, the exponent, or logarithm, is a negative one, yet it is more convenient to express acidity as a positive value. This is done by using the logarithm of the reciprocal of the hydrogen-ion concentration; this logarithm is known as the *pH* of the solution. *H* stands for hydrogen, and *p* for "Potenz" (Ger., power), *i.e.*, the exponent, or logarithm. The pH of a solution is, therefore, the exponent, or logarithm, of the reciprocal of the hydrogen-ion concentration.

$$\text{pH} = \log \frac{1}{[\text{H}^+]}$$

If, now, hydrogen-ion values ranging from 1.0 to 10^{-14} are expressed on the same scale mentioned above, but this time in terms of pH, a sheet of paper only 14 in. long is needed, and very cumbersome mathematics is avoided, though some inaccuracy is thereby introduced.

Decreasing acidity (10^{-5} , 10^{-6} , 10^{-7} , etc.) means increasing pH (5, 6, 7, etc.). This is unfortunate and confusing, though more troublesome to the beginner than to the advanced worker, who soon gets into the habit of thinking in terms of pH. Wherry has tried to simplify the matter for the nonchemical investigator by calling the neutral point of pure water (pH 7) unity; then the "specific acidity" of a solution having a pH of 6 is 10, which is the actual relative concentration of the hydrogen ions, *i.e.*, ten times as great as in pure water. Lemon juice, with a pH of about 2 (the pH of *N/100 HCl* is 2) has, on this basis, a "specific acidity" of 100,000 (times that of water); and sea water, with a pH of 8.2, has a "specific alkalinity" of slightly over 10.

But there is a greater difficulty which often leads to a faulty conception of the magnitude of a change in acidity. The difference between pH 4 and 5, 5 and 6, and 6 and 7 (the logarithms of the hydrogen-ion concentrations) is in each case 1, but the difference in the actual concentrations of hydrogen ions is in each case ten times that of the preceding value, which

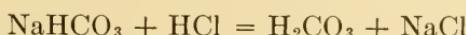
means that pH 4 is, in actual concentration of hydrogen ions, one thousand times as great as pH 7. Furthermore, in comparing pH values and in making averages, actual hydrogen-ion concentration values must be used and not the logarithms of these values, *i.e.*, not pH values. The same should really be done when plotting curves, if a true picture is to be given, but the values become cumbersome. Striking and significant differences are sometimes obtained when a curve is plotted on the basis of pH values and then plotted for the same experiment on the basis of actual hydrogen-ion concentrations. The error in using the average of several pH values instead of the pH value of the average of the hydrogen-ion concentrations is illustrated in the following example. The average of pH 5 and 6 is pH 5.5, but the pH of the average of the two hydrogen-ion concentrations for which pH 5 and 6 stand is pH 5.3. Obviously, the latter is the correct value to use.

As there is a corresponding hydroxyl ion concentration for every hydrogen-ion concentration, alkalinity as well as acidity may be expressed in terms of pH. Hydroxyl ion concentration could be expressed in terms of pOH as well, but pH serves the same purpose. The following table gives a series of pH values and the corresponding actual H^+ and OH^- concentrations in molecules per liter, which, as each H^+ ion weighs 1, become, in

Normality	pH	H^+ , moles/l.	OH^- , moles/l.
<i>N</i> HCl.....	0.0	1	10^{-14}
0.1 HCl.....	1.0	10^{-1}	10^{-13}
0.01 HCl.....	2.0	10^{-2}	10^{-12}
0.001 HCl.....	3.0	10^{-3}	10^{-11}
0.0001 HCl.....	4.0	10^{-4}	10^{-10}
0.00001 HCl.....	5.0	10^{-5}	10^{-9}
0.000001 HCl.....	6.0	10^{-6}	10^{-8}
Neutrality.....	7.0	10^{-7}	10^{-7}
0.000001 NaOH.....	8.0	10^{-8}	10^{-6}
0.00001 NaOH.....	9.0	10^{-9}	10^{-5}
0.0001 NaOH.....	10.0	10^{-10}	10^{-4}
0.001 NaOH.....	11.0	10^{-11}	10^{-3}
0.01 NaOH.....	12.0	10^{-12}	10^{-2}
0.1 NaOH.....	13.0	10^{-13}	10^{-1}
<i>N</i> NaOH.....	14.0	10^{-14}	1

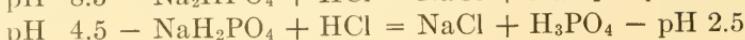
terms of molarity, grams per liter for hydrogen. The OH⁻ ion concentration is also expressed in molecules per liter, or equivalent concentrations; the weight in grams would then be obtained by multiplying by 17, the weight of the OH⁻ ion.

Buffers.—Pure water is neutral with a pH of 7. If 1 cc. of 0.01*N* HCl is added to a liter of water of pH 7, the resulting pH will be 5; but if this amount of acid is added to a beef infusion of pH 7, the change in pH will be hardly noticeable. This power of solutions to resist a change in pH was likened to a *tampon*, or swab, which soaks up a fluid—not an especially good analogy. *Puffer* in German and “buffer” in English are both from Old French *buffe*. (The French use the word *tampon* for buffer.) A better analogy is that between a pH buffer and a mechanical buffer (a blow or bump) both of which ease a shock. Any substance that prevents a sudden or pronounced change in acidity is a buffer. All physiological solutions such as blood, milk, and sea water are buffered. Buffers are extensively used in the laboratory to maintain solutions at a constant pH value. There are a variety of buffer salts, a well-known one being acetate. The acetate itself is nearly neutral. If strong hydrochloric acid is added to it, weak acetic acid and neutral sodium chloride are produced. Sodium bicarbonate is another buffer salt and one of great biological importance. Blood is well buffered by bicarbonate. It acts in the following manner:



in which the strongly dissociated hydrochloric acid has been converted by the buffer into the weakly dissociated carbonic acid, the pH change from that of the original buffered solution being small.

The behavior of sodium phosphate salts is a good example of buffer action. If to a highly alkaline solution of trisodium phosphate of pH 10.5 an equivalent amount of strong hydrochloric acid of pH 1 is added, then alkaline disodium phosphate and neutral sodium chloride result. More strong acid of pH 1 will bring the solution down to only pH 4.5. Still more acid of equivalent amount will finally bring the mixture to pH 2.5. The sodium chloride formed each time is neutral.



Buffers are usually prepared as mixtures of two salts, a well-known buffer pair being acetic acid and sodium acetate. If these two salts are mixed in different proportions, and the pH values of the mixtures plotted, the curve shown in Fig. 144 is obtained.

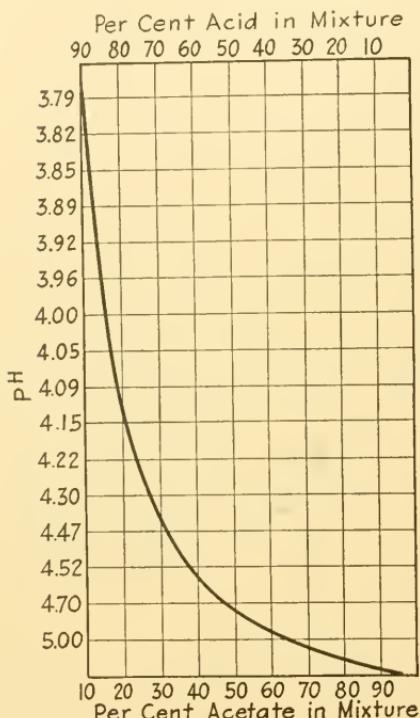


FIG. 144.—The hydrogen-ion concentration of mixtures of acetic acid and sodium acetate.

If hydrochloric acid in not too great quantity is added to any of these mixtures, the pH remains fairly constant. The reason is as follows. The dissociation of acetic acid is slight ($\text{CH}_3\text{COOH} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{H}^+$). The dissociation of sodium acetate is great ($\text{CH}_3\text{COONa} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{Na}^+$), as is also that of hydrochloric acid ($\text{HCl} \rightleftharpoons \text{H}^+ + \text{Cl}^-$). The following ions, therefore, occur in a solution of these three substances: CH_3COO^- , Na^+ , H^+ , and Cl^- . The fact that acetic acid ionizes to only a very slight degree means that CH_3COO^- and H^+ ions cannot exist together in solution to a

very great extent and, therefore, that the larger part of the H^+ ions from the hydrochloric acid added will immediately combine with CH_3COO^- ions to form undissociated molecules of acetic acid. The concentration of acetic acid has thus been somewhat increased, but as acetic acid dissociates to such a slight degree, a small increase in its concentration does not change the hydrogen-ion concentration of the solution very much. While a pH determination would indicate little change in hydrogen-ion concentration, titration would show the total amount of acid added (total

replaceable hydrogen atoms). A pronounced change in normality (titratable acidity) of the buffer mixture represents a very slight change in pH.

A similar state of affairs exists when an alkali is added to an acetic acid and sodium acetate buffer. The alkali, *e.g.*, sodium hydroxide, will dissociate into Na^+ and OH^- ions. Water is only slightly dissociated; few H^+ and OH^- ions can, therefore, exist together in an aqueous solution. The OH^- ions from the sodium hydroxide combine with the H^+ ions to form water. This will rob the acetic acid equilibrium $\text{CH}_3\text{COOH} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{H}^+$ of some of its H^+ ions and leave unbalanced CH_3COO^- ions, which are then taken care of by the free Na^+ ions from the sodium hydroxide to form more sodium acetate. The acetic acid dissociation constant K must be maintained; consequently, more of the acetic acid will dissociate. K is now constant again, with a slight decrease in total concentration of acetic acid, which does not appreciably affect the pH value but which titration will show to have taken place.

It is thus evident that considerable quantities of acids or alkalies may be added to solutions containing buffer salts without materially changing their pH values.

METHODS OF MEASURING pH

Color Indicators.—The chemist has long used litmus as a color indicator of the acid or alkaline state of a solution. Litmus when acid is red; when alkaline, blue. At best, it indicates simply whether the solution is neutral, slightly acid or alkaline, or strongly acid or alkaline. Litmus is of plant origin, coming from *Roccella tinctoria* and other lichens. Cochineal, from the insect of that name, is an indicator of animal origin which has also been used for acidity determinations.

An interesting example of the indication of pH by color is that of flowers the color of which is due to acidity values of the particular pigments that they contain. The change in the color of flowers as they open and wither, and probably of leaves in autumn, is due to change in pH. The pigments may be extracted and used as indicators. Anthocyanin (the soluble pigment of red and blue flowers) from the tulip has a pH range of 5.3 to 9.2. One of the earliest references to natural pigments as acid-alkaline indicators is that of Robert Boyle, who, in 1664, called attention

to "syrup of violets" as a color indicator. He expressed particularly his surprise and admiration on learning that not only did "spirit either of salt or vinegar, or almost any other eminently acid liquor" turn the syrup of violets red but "a little oil of tartar *per deliquium*, or the like quantity of solution of pot-ashes" turned the syrup into a perfect green. More recently, blueberry and red-cabbage juice have been used. Red-cabbage juice gives a good range in color changes from deep red to blue-green. The range is as follows:

pH	Color	pH	Color
1	Bright rose	6	Blue
2	Crimson	7	Green-blue
3	Red lilac	8	Blue-green
4	Magenta	>8	Brilliant green to green-yellow
5	Purple		

Willstätter noted that anthocyanin is responsible for both the color of the rose and that of the cornflower; the colors are different, because the former is at a pH of 5.5 while the latter is 7.2. E. Philip Smith found the anthocyanin pigments of a number of flowers to show in test tubes colors comparable to those in the living petals, so that by matching the petal with the pH color standards, the pH of the cell sap can be estimated. The flowers examined showed pH values of 3.1 to 7.7. The red group is acid, and the blue alkaline. The method allows observing pH changes during opening and withering and the effect of environmental conditions on flowers.

There are also yellow pigments in plants which serve as pH indicators both within and without the plant. A number of these have been calibrated, including that from the plasmodium of the slime mold *Physarum polycephalum*. This pigment, which is possibly a flavone (page 517), has a color and pH range as follows:

pH	Color	pH	Color
<1	Deep red-orange	5	Yellow
1.5	Red orange	6	Lemon yellow
2	Deep orange	7	Sulphur
3	Orange	8	Yellow-green
4	Yellow-orange	>8	Green

Wine manufacturers have long titrated red wine for total acidity, using the color change from red to green as an end point. The pigment which thus serves as an indicator is in the grape skin.

Such indicators serve well enough for crude determinations; but in laboratory physiological work, where hydrogen ions play so prominent a role, more precise measurements are necessary. Two synthetic indicators, methyl red and phenolphthalein, had begun to take the place of litmus as acid-alkaline indicators before the now more commonly used indicators were introduced. Methyl red and phenolphthalein are sufficient for most titrometric (normality) purposes, but their pH range is not great. Through the efforts, first, of Friedenthal and Salm, then of Sørensen in Copenhagen, many dyes that change their color with change in acidity of the solution were carefully studied, and their accuracy as pH indicators determined by comparison with other (electrometric) methods of pH determination. Nine dyes thus selected indicate a pH range from 1.2 to 9.6. These indicators are synthetically produced from coal-tar products. One of them, bromphenol blue, is yellow at a pH of 3.1 or less and blue at a pH of 4.7 or more. If the pH of the solution is above 4.7 or below 3.1 the dye next in the series of color changes must be used.

Dyes change color with change in acidity, because they dissociate into a colored ion, with a noncolored or different-colored ion. They do so as a simple salt (phenolphthalein is a sodium salt) or, less often, as *ampholytes*, *i.e.*, *amphoteric* substances (see page 483). In both cases, the end result is the same—an ion with a color is liberated on dissociation. The degree of dissociation, and therefore the concentration of the colored ion, depends on the hydrogen (or hydroxyl) ion concentration. A change in pH consequently means a change in the color of the solution. Thiel has shown that the color changes rest upon more complex reactions, involving molecules, simple ions, ions of different valence, "Zwitter" (double) ions, and isomeric changes.

Colorimetric pH determinations are made in a *colorimeter*, or *comparator*, which may be of the LaMotte roulette-wheel type. The color of a solution of unknown pH to which an indicator dye has been added is compared with that of a standard solution of known pH and color.

The first pH color indicators were plant products, and now plants again make their contribution to the list of indicators. Pratt and Swartout investigated pigments of fruits and vegetables and found that apricots, carrots, peaches, pears, persimmons, and tomatoes failed to yield pigments with indicator characteristics, while red apples, a variety of berries, black cherries, grapes of all colors from red to black, certain plums, pomegranates, and prickly pear (cactus) fruit proved to contain pigments of indicator value. The pH range of several of these pigments is given in the following table:

Fruit sources	Color change	pH range
Apples.....	Red to yellowish green	6.2 to 7.2
Blackberries.....	Red to dark grayish blue	6.0 to 7.4
Cactus.....	Red to faint purple	9.0 to 12.0
Cranberries.....	Red to yellowish green	6.2 to 7.2
Grapes.....	Red to purple	5.0 to 6.6
Grapes.....	Purple to green	6.6 to 7.6
Pomegranates.....	Red to purple	6.0 to 6.8
Pomegranates.....	Purple to green	6.8 to 7.6
Strawberries.....	Red to yellowish green	6.2 to 7.2

Concentration vs. Activity.—Acidity in terms of hydrogen ions, or pH, is usually defined and expressed as a concentration. When Sørensen recommended the symbol pH^+ , which has since become pH, he thought that he was measuring $\text{pH} = -\log \text{CH}^+$, *i.e.*, the negative logarithm of the hydrogen-ion *concentration*. That concentration is a factor is evident from the fact that with increase or decrease in concentration of an acid or an alkali, a different pH value results; but while it is still convenient to express pH as a quantity factor, what we actually measure (by color or electrometrically) is *activity*; therefore, instead of pH being an expression of concentration, it is an expression of activity ($a\text{H}^+$), and $\text{pH} = \log \frac{1}{a\text{H}^+}$. (The true situation is a little different from that as just stated; we measure neither concentration nor actual activity but activity with standards based on concentration, owing to a theory that appears invalid today but was acceptable when first expressed by Sørensen on the basis of the then existing ionization theory of Arrhenius.)

While considerable confusion has resulted, the situation is not so bad as it seems, for there is a definite relation between concentration and what we are actually measuring; furthermore, the numerical differences are not great. The relationship between concentration and activity is shown in the following formula:

$$a = Cf$$

where a is the activity; C , the concentration; and f , the activity coefficient, which varies for different ions and different concentrations. The expression a = activity, or *effective* concentration, indicates the relationship further. Kilpatrick recommends that the definition based upon concentration be maintained, for pH represents the logarithm of a quantity, and in many cases a knowledge of the concentration of the hydrogen ion is more important than a knowledge of its activity.

The Potentiometer.—If two solutions of different ionic concentrations, whether containing hydrogen or not, are brought into contact conveniently by what is known as an agar bridge, which is a glass U tube containing a salt solution held in jelly (Fig. 152), then the difference in potential between them can be measured. What we have is a galvanic chain, just as exists in an electric (Daniell's) cell. A potential is set up at each electrode because of the reaction between the metal and the surrounding electrolytic solution. The difference in the potentials of the two electrodes may be measured and expressed in volts. If one of the electrodes is sensitive to hydrogen ions, then the potential difference established is a measure of the pressure or activity of the hydrogen ions. The potential can be determined by opposing it with a known electromotive force (e.m.f.) which is just sufficient to prevent a flow of current. That a condition of no flow has been reached is ascertained by the absence of deflection of a galvanometer.

The German physicist Poggendorff introduced the *compensation*, or *potentiometric*, method for measuring electromotive force with the aid of a *potentiometer*, so called because potentials are measured. The potentiometer of today includes most of the parts and a number of accessory ones that were formerly placed out on the laboratory table. In Poggendorff's day, the potentiometer was a "setup" rather than a single piece of apparatus.

The potentiometer is an instrument for comparing an unknown potential with a known one, without drawing current from the unknown. A known and an unknown electromotive force are connected so that the known force E (Fig. 145) is joined to the two ends A and B of a wire of uniform resistance per unit length, and the unknown force E' connected so that one or both of its terminals C and D are movable along the wire. A galvanometer G is inserted in the circuit with the unknown e.m.f. E' . (R and S in Fig. 145 are for the moment ignored.) The known e.m.f. E may be a voltaic cell of about 2 volts; the unknown is the feeble potential set up by free hydrogen ions in a solution. The e.m.f. of each of these two systems (the upper one M and the lower one N) will oppose each other if like poles of E and E' are connected. If the two currents (of M and N) oppose each other, then there will be no flow through N in either direction when the potential

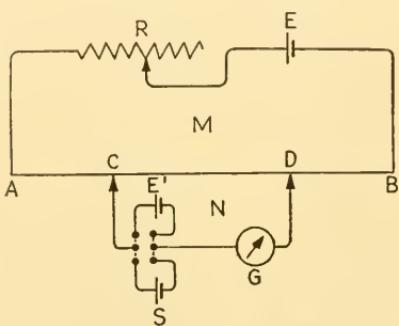


FIG. 145.—Diagram illustrating the principle of the potentiometer.

drop between C and D is equal (and opposite) to the potential of E' . At this point, there will be no deflection of the galvanometer. Once the position of C and D is determined, at which point the galvanometer shows no deflection and $CD = E'$, then it is necessary only to ascertain what part CD is of the total length (potential drop) of AB , that is to say, what part E' is of the known potential E . If the unknown potential E' is a part of E , then, naturally, E must always be larger than E' . The calculation is made from the ratio $E:E';AB:CD$, from which

$$E' = \frac{CD}{AB} \times E.$$

Whatever we now add to our Poggendorff equipment has nothing to do with the fundamental principle involved but adds only to the refinement of the method. The source of current E must be of a known and a constant potential, which is never true of the usual electric cell. E must, therefore, be calibrated and frequently checked against a very accurate *standard cell*

(*S*, Fig. 145). Such a cell has been devised by Weston. It is an H-formed vessel containing cadmium and mercury and has been adopted as the international normal electrical element, or standard cell, of voltage 1.0183. The voltage remains very constant. Similar is the Eppley cell. The cell is not used permanently but just long enough to calibrate the source of electromotive force. In an experiment, the known electromotive force E is first balanced against the standard cell S by adjusting resistance R included in the circuit. To do this, the unknown electromotive force E' is disconnected, and the standard cell S inserted in its place. When the system M (with R) is balanced with system N (with S), then the galvanometer will show no deflection, and the voltage drop from A to B is equal to the voltage of the standard cell, or 1.0183 volts. All that has been done is to make the voltage of system M , *i.e.*, of E when in series with R , equal to a definite and accurately known potential, *viz.*, that of the standard cell S . This done, that is to say, with system M calibrated, S is disconnected, and E' reconnected and balanced with the system M as before. The formula then becomes

$$E' = \frac{CD}{AB} \times 1.0183 \text{ volts}$$

If AB is of 1,000 units of length (1 m.), then 1 unit (1 mm.) is equal to 0.0010183 volt (or 1 mv.), because the total drop from A to B is 1.0183 volts, the voltage of the standard cell; consequently, the potential drop from C to D can be read directly.

Most of the above-mentioned parts of the Poggendorff setup are, with others, put into the modern "type-K" potentiometer of Leeds and Northrup, illustrated in Fig. 146; the inner connections are shown in Fig. 147.

If we are concerned with the potential produced by hydrogen ions, we must have a hydrogen electrode, which simply means an electrode sensitive to hydrogen ions. This may be made by coating a noble metal, such as platinum, with spongy (colloidal) platinum or palladium and then saturating the electrode with hydrogen. Platinum is known to have the property of holding hydrogen; the minute pores of the spongy coating, greatly increase the surface and therefore the adsorption of hydrogen ions. The spongy platinum when saturated with hydrogen gas

becomes a hydrogen electrode, *i.e.*, an electrode of hydrogen gas sensitive to hydrogen. To keep the electrode saturated, a

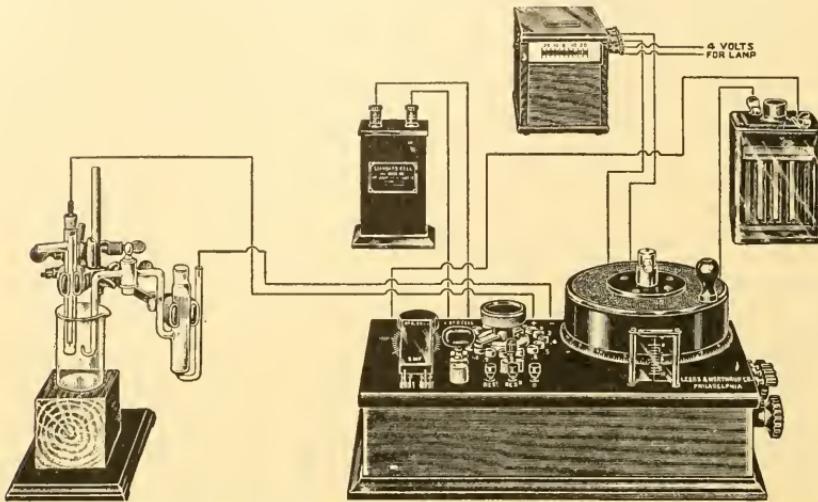


FIG. 146.—Connections for making pH determinations with a Leeds and Northrup Type-K potentiometer.

stream of hydrogen gas must be constantly flowing through the solution (the gas does not affect the pH of the solution).

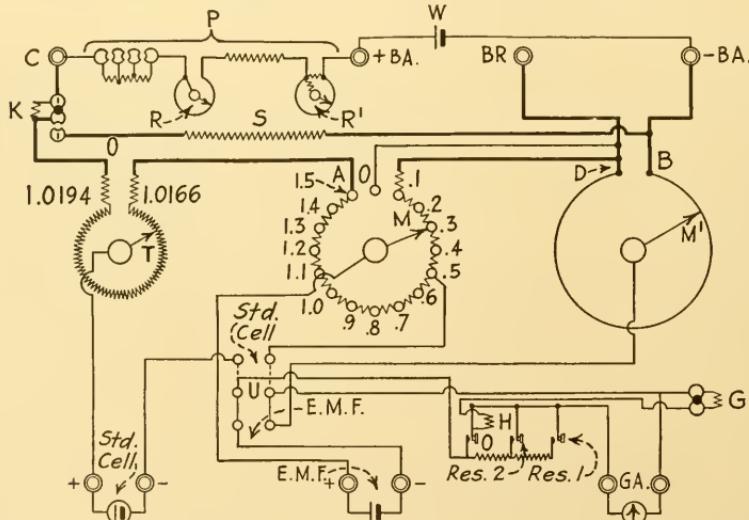


FIG. 147.—Interior connections of Leeds and Northrup Type-K potentiometer.

It is not possible to measure the potential of a single electrode. Two electrodes are necessary to give a measurable potential.

If one is known, and the difference measured, then the other can be calculated (by the formula of Nernst). That electrode of which the potential is known is termed the *reference electrode*. In the case of hydrogen cells, the reference electrode may be made by immersing a hydrogen electrode in a buffer solution of known pH value. The other electrode is immersed in the solution of unknown acidity. The two are connected through an instrument for measuring potential (the potentiometer), the solutions

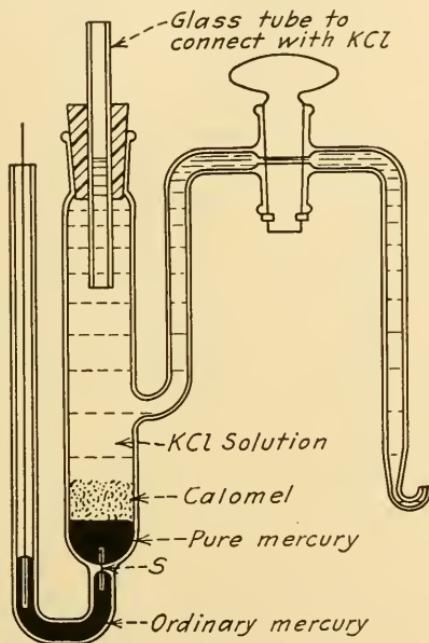


FIG. 148.—Calomel cell.

themselves being joined by an agar bridge. The original standard hydrogen reference electrode was one of hydrogen gas immersed in a solution that is normal in respect to hydrogen ions at one atmosphere pressure. At present, the *calomel electrode* is the standard reference electrode. It is a glass tube containing a potassium chloride solution saturated with and resting on mercurous chloride (calomel) and mercury (Fig. 148). It and the hydrogen electrode are called *half cells*. The two constitute a complete cell. The potential difference between them is measured. The potential of the calomel cell must, obviously, be known for reference. If it contains normal potassium chloride,

its potential is 0.2848 volt at 25°C. The following equation (based on the Nernst formula) gives the relationship between the potential difference measured and the hydrogen-ion concentration of a solution:

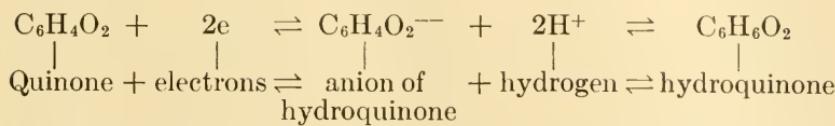
$$\text{pH} = \frac{E_x - E \text{ (calomel)}}{0.0591} \text{ at } 25^\circ\text{C}.$$

In practice, as the wire is so calibrated that a thousandth part is equal to one millivolt, it is necessary only to read the wire length (*CD*, Fig. 145), which gives directly, by its length, the potential difference in millivolts between the calomel and hydrogen electrodes. A table converts the potential difference into a pH value. A wire length of 567.6 mm. represents a voltage of 0.5676, which, at 15°C., indicates a pH value of 5.5; a voltage of 0.6819, at the same temperature, indicates a pH of 7.5.

The Quinhydrone Method.—The quinhydrone method in its simplest form involves balancing the potential produced by quinhydrone in a solution of unknown pH with that produced by quinhydrone in a buffer mixture of known pH. Quinhydrone, through the addition of water, breaks down to hydroquinone and quinone. (These last two substances could be used separately in proper proportions; it is simply more convenient to start with quinhydrone.) An equilibrium is established between the hydroquinone and quinone, resulting in the giving off of two ions of hydrogen and two electrons. The two electrons produce the potential which is measured, or, rather, there is a difference in the level of free energy which is utilized by means of platinum electrodes; an electric current, with a difference in potential, results. Zero deflection of the galvanometer in the circuit indicates that the potentials, and therefore the hydrogen-ion concentrations, are the same in both solutions.

Quinhydrone yields equimolecular concentrations of hydroquinone which is a reductant and quinone which is an oxidant. What is actually being measured in the quinhydrone method of pH determination, is an electromotive force which has a definite relation to the free energy produced in a chemical reaction, in this case brought about by the reversible process of an oxidation-reduction equilibrium. The rate of the reaction, and therefore the potential, is determined by the hydrogen-ion concentration

of the solution. A possible interpretation of the complete reaction is:



The method proceeds as follows. An "unattackable" metal such as gold or platinum serves for the electrodes. No reference (calomel) cell is needed, as a potential is not being measured; instead, two potentials, a known and an unknown one, are balanced. A small quantity of quinhydrone is added to both solutions, the unknown and the known (the buffer mixture), each in small beakers connected by an agar-salt (potassium chloride) bridge. One member of the buffer pair is placed in its beaker, and to it the other buffer is added drop by drop from a burette until a potential (H^+ ion concentration) is obtained which balances the potential of the unknown in the other beaker, as indicated by a zero reading of the galvanometer. With the two systems in balance, then the pH of the unknown is obviously equal to that of the buffer mixture which is determined from the proportions of its two constituents.

The quinhydrone method may be combined with the potentiometric setup, as described for the hydrogen electrode. All connections remain the same (Fig. 143). No buffers are then used, as the potential is measured directly. A reference (calomel) cell is now needed. The unknown solution with quinhydrone and a metal electrode take the place of the hydrogen electrode.

The quinhydrone method has replaced others in certain types of research, particularly commercial work, as in the manufacture of food products. But it has its limitations. It is not good for pH values above 8.5. It is inaccurate for whole-blood determinations owing to a reaction between hemoglobin and quinhydrone, though fairly accurate for blood serum and plasma.

The Electrometer.—Instead of a galvanometer, an electrometer may be used with the hydrogen electrode or with quinhydrone. Of the two chief types, the quadrant electrometer is the more sensitive (it is a very sensitive instrument). The capillary electrometer is less delicate but very satisfactory and has the advantage of being simple enough to be made in the laboratory,

a practice more common in European laboratories, where cost has always been a greater factor than in America. Both instruments, like the galvanometer, are used in pH work, not actually to measure potential but simply as instruments to indicate the "null" point, or point of no flow of current—the point of equilibrium. The capillary electrometer operates on the principle that the surface tension of mercury, when in contact with an electrolyte (dilute sulphuric acid), is a function of potential. The potential existing at the interphase between the mercury and the electrolyte determines the surface tension of the mercury, which, accordingly, rises and falls in its capillary tube. The change in surface tension is produced by a change in potential. While not ordinarily used as such, the capillary electrometer is a means of measuring potential directly; it is sensitive to 0.0001 volt.

The Glass and Other Hydrogen Electrodes.—A number of electrodes for measuring hydrogen and other ions have been developed. Among the most important of these is the glass electrode for hydrogen-ion determination. It has given especially reliable results on the pH of blood, a material that has always presented difficulties because of temperature changes and the giving off of carbon dioxide when the blood is withdrawn and exposed to the air. The glass electrode was introduced by the German physical chemist Fritz Haber.

When we say that the glass electrode is a hydrogen electrode, we have not said quite the same thing as when the statement is applied to a hydrogen electrode of platinum metal coated with platinum black and saturated in hydrogen gas, because this latter electrode is, in part, of the element hydrogen. This is not true of the glass, antimony, and other electrodes. All are hydrogen electrodes in the sense that they measure hydrogen ions. The potential difference between the sides of a film of glass when immersed in liquid is affected by hydrogen ions, so that a measure of the former is a measure of the latter.

The glass electrode is an exceedingly thin membrane of glass, either in the form of a blown-glass bulb or of a glass film sealed on to a tube. Into this tube a convenient electrolytic solution is put ($0.1N$ HCl), and the tube immersed in the solution of unknown pH. A calomel half cell forms the other electrode. The thin membrane of glass may be regarded as a condenser. Between its two sides is established a difference in potential which is pro-

portional to the hydrogen-ion concentration. The current is so very slight, owing to the high resistance across the glass (often as much as 30 megohms, or 30,000,000 ohms) that it must be measured by a supersensitive instrument or be amplified. For the first of these methods, a quadrant electrometer is used, and, for the second, vacuum-tube amplifiers increase the current to the point where it can be measured on an ordinary galvanometer. In more recent models, where the glass is made very thin, the resistance is low enough to yield a potential that can be read with an ordinary galvanometer and potentiometer without amplification. The glass for the electrode must be a very pure calcium-sodium-silicate glass. Lime, soda, and silica are melted together to produce it. The slightest trace of lead or aluminum is fatal.

Errors.—Errors enough creep into pH technique, but some can be systematically determined and usually allowed for. These are the salt, protein, and temperature errors.

Salt effects cause a displacement of the apparent pH, which results in a shift in the color of the indicator. The following table gives the correction in pH for certain salt concentrations:

Indicator	Salt	Salt concentration	Correction
Methyl orange.....	KCl	0.10 N	-0.08
Methyl orange.....	KCl	0.50 N	+0.02
Methyl orange.....	KCl	1.00 N	+0.23
Phenol red.....	NaCl	0.50 N	-0.15
Phenolphthalein.....	NaCl	0.50 N	-0.17

The protein error is, in general, very small, unless the concentration of the protein is exceedingly great, as the following table shows:

Globulin, per cent	Phenol red, pH deviation	Albumin, per cent	Phenol red, pH deviation
2	0.00	0.03	0.00
4	+0.02	0.06	-0.02
12	+0.04	0.13	-0.03

It is difficult to correct for the temperature effect on pH, as the coefficient is apparently quite variable, although the change in blood measurements due to it is now fairly well established. Measurements of the pH of blood should be made at 38°C. If made at a lower (room) temperature, the pH changes 0.01 for every degree, which means that with lower temperature blood becomes more alkaline. Normal blood has a pH of 7.35 to 7.43 at 38°C., with 7.38 the usual value. At 20°C., it will be about 7.55.

The lipoid error can at times be very great and is not easily corrected. Also not readily allowed for are the color errors in colloidal systems. Deutsch has shown that pH indicators do not give the correct colors in systems with great adsorptive surfaces such as colloidal solutions, gels, and the like. There is a displacement of the pH equilibrium and adsorption of the dye, resulting in colors from those quite different in homogeneous solutions. (This is probably true only of those dyes the undissociated form of which is lipoid soluble and not of amphoteric dyes.)

The Role of Hydrogen Ions in Life.—The part that hydrogen ions play in vital processes is one of the outstanding biological problems of today. Perhaps we have deified the hydrogen ion, but there is every reason to believe that while later work may take away from hydrogen some of the significance that we are now attaching to it, it will always remain one of the most important ions in physiological reactions. Aside from its acid properties, its minimum size and maximum speed place it in a unique position among ions.

The role of hydrogen in life is so great and so widespread that one can hardly mention a process of an organic nature that is not influenced by these ions. Their effect in the nonliving world is probably no less extensive. Leather tanning, breadmaking, brewing, aging of wines, tea curing, fermentation reactions, electroplating, chemical precipitations, the settling of muddy water, and the productiveness of soil are all properties the rate and success of which depend upon pH.

Soil problems play an important role in agriculture and in natural plant distribution. They involve the influence of acidity on soil reactions, plant reactions, and the relationship between plant and soil, such as the availability of soil nutrients to the

plant. One interesting phase of the problem is the relation between the acidity of the soil and the kind of plant that grows in it. There is a tradition among farmers that moss or sorrel, the red-flowered *Rumex acetosella*, or yellow-flowered *Oxalis stricta* in a field is a sure sign that the soil is "sour," but the studies of Wherry involving many tests of hydrogen-ion concentration show that these plants do not correlate with high acidity at all. The soil reaction may be alkaline, to pH 8, even where the growth of sorrel is luxuriant. These plants indicate sterility, not acidity. However, other plants, e.g., bluets (*Houstonia caerulea*), are very rare on neutral soil and almost always appear in a field where the rain has leached out most of the surface alkalies and an acid condition is beginning to develop.

Chlorosis of plants, a disease involving loss of chlorophyll and resulting discoloration of the leaves from the normal green to a sickly yellow, has long been known to be due to a deficiency in iron, which functions in the plant as a catalyst in the manufacturing of chlorophyll. To supply this deficiency, plants were sprayed with iron chloride, and the disease disappeared. But it was later found that there is usually sufficient iron in the soil and even in the pale yellow leaves themselves—indeed, chlorotic leaves sometimes have more iron in them than normal ones. Apparently, therefore, some factor other than iron deficiency is, or may be, the cause, yet spraying with iron chloride does help. It was observed that chlorotic plants usually grow upon alkaline soil. Nowhere is this better seen than in the Crimea, where the soil is highly alkaline, and chlorosis in fruit trees is very common. The natural conclusion is that alkalinity and not iron deficiency is usually the cause of chlorosis. Adding acid, and not iron, to the soil or leaves is, therefore, the correct thing to do. Addition of acid makes the iron available to the plant. Spraying with iron chloride does not add needed iron but simply makes the leaves more acid and thus makes the iron, already present in sufficient quantity, available. While this is perfectly true, it is not the whole story.

Chlorosis of plants may occur on neutral soil where the disease is a pure case of iron deficiency. On the other hand, there may be an absence of chlorosis on alkaline soil (where plants are usually chlorotic). The healthy plant succeeds here because the iron is available not as a salt like iron chloride, as is ordinarily

true, but in an organic form. In this case, alkalinity does not determine the availability of the iron for the plant.

The constancy with which soils hold their pH value is not very great. Some soils are well buffered with an ammonium silicate complex, and others not; the former maintain a fairly even pH value.

More uniform in its acidity is the ocean, with an average pH of about 8.2, but even it is not absolutely constant in any one locality, and it varies considerably between points. The coral shores of Bermuda make the water there more alkaline than that off the rock shores of Maine. Plant and animal life in the ocean are nicely adjusted to the pH of their natural habitat. The green alga *Valonia*, collected in Bermuda, will not grow well in the waters of Massachusetts Bay (salt concentration or temperature, as well as pH, may be responsible here).

While organisms seem to be very exacting in their external pH requirements, they also appear, in cases, to be rather indifferent to it. Mast found that *Amoeba proteus* can live in a surprisingly wide range of hydrogen-ion concentration. Some specimens lived eight days in pH 3.8 to 4.2, and others seven days in pH 8.2 to 7.8. In concentrations between these, there is no pronounced difference in the length of life. Beyond pH 4.2 and 8.2, the length of life decreases very rapidly.

Olof Arrhenius, son of the great Svante Arrhenius, author of the dissociation theory, has aroused much interest and confidence in pH as applied to agriculture in his country and in Germany—so much so that a farmer in Germany will hesitate to grow neutral-soil plants on an acid soil (liming being less depended upon there than in the United States). For example, sugar beets have their highest yield in sugar content on neutral soil (pH 7). Farmers are, therefore, discouraged from risking a crop of sugar beets on an acid or alkaline soil. In the main, this is a good policy, but the theory does not always hold. It may be simply a question of the way in which a plant wants its nitrogen, and pH may determine the form in which the nitrogen occurs; but if it is possible to give the plant its nitrogen in the desired form regardless of the pH value of the soil, then the plant becomes indifferent to pH (within limits), and measuring the pH of the soil may then tell nothing of significance. Acid-soil plants, such as members of the heath family (*e.g.*, rhododendron), prefer their nitrogen

as ammonia or amines, while neutral-soil plants, such as ordinary garden and crop plants, prefer theirs as the nitrate ion.

Acidity has come to play a very important part in medicine. How far changes in acidity are the cause and how far the result of disease is a debated question, with the majority holding the latter view.

The first problem to consider is the pH of the blood. Blood is well buffered and therefore maintains a very constant pH value which, normally, is about 7.4 (*i.e.*, slightly alkaline). Difficulty in measuring the pH of the blood has been the cause of much uncertainty and some controversy over values. The first errors enter when the blood is drawn off. The pH value determined several minutes later may not be very near the value that exists in the blood stream. The carbon dioxide tension of the blood is higher than that of the air; therefore there is a loss of carbon dioxide when blood passes through air. If the loss of carbon dioxide is partially prevented by drawing the blood off under oil, there are still the errors due to drop in temperature and the presence of proteins. The effect of the loss of carbon dioxide from the blood stream is interestingly and convincingly illustrated in the faint condition which results in human beings from excessive heavy breathing. This may go so far as to cause unconsciousness. It is due to an abnormally high alkaline state produced by loss of carbon dioxide.

While measurements of blood pH may involve many errors, the values obtained appear to be fairly accurate, and at least comparable, as the same errors creep into all determinations when made by one method. It is now generally conceded that for blood determinations the colorimetric method is the most reliable as well as the most rapid and convenient.

Other body fluids have their characteristic pH values. Gastric juice may be very acid with a pH of 1.0 to 6.0, usually about 2.0. (Lemon juice is about pH 2.) Urine is 4.8 to 8.2, with 6.0 as the average (this is the pH range of phosphate buffers, which probably maintain the pH of the blood). Saliva may be slightly acid (6.8) though the mouth is usually alkaline. Cerebrospinal fluid is just above neutrality, with a pH close to that of blood—7.3 to 7.4 (for unknown reasons, the cerebrospinal fluid is very poorly buffered).

If blood is well buffered, there should be little change in acidity. How much may it, therefore, change with change in health? This is a debated question. The evidence seems overwhelmingly to support the statement that blood pH is not constant at all times and under all conditions, yet some believe that disease (*e.g.*, cancer) involves no significant change in pH. Others claim to have observed a change (to the alkaline) so marked (pH 7.44) as to aggravate the disease. Probably both conditions exist, depending upon the disease and the patient. Blood is certainly well buffered but not against all conditions.

The blood stream helps maintain an equilibrium in chemical reactions in the body by keeping temperature constant and taking care of a too acid or too alkaline condition, which it can do because of its own highly buffered state and generally uniform pH value. The blood maintains this uniform pH value by adjustment between the red cells and the plasma. The red blood cells take up acid and give off base to the plasma. In passing through the lungs, they lose carbon dioxide and thus regain their pH value. Naturally, other body functions materially contribute toward maintaining an acid-alkali equilibrium. The formation of acid in the body is compensated for by the excretion of acid in urine, the formation of ammonia, and the elimination of carbon dioxide through the lungs. As long as these reactions are coordinated, the blood is of constant pH, and all is well.

MacDonald has shown that there is considerable evidence to indicate a marked increase in the hydrogen-ion concentration of the blood during fright, anger, anesthesia, and shock. It is good medical technique to draw blood from an experimental animal when it is in a quiet state and contented. The pH of the blood of a frightened or excited animal is likely to be abnormal. In practice, the animal should not even quiver from nervous fear. It is maintained that a man in a high state of emotion (from fear) has a low blood pH value (7.25), while when he is quiet and calm, his blood may, a day later, rise to a pH of 7.42. Hysteria and dilated pupils appear to be associated with alkalosis, and psychasthenia with acidosis. According to Cannon, hunger, fear, pain, etc., cause a diversion of blood from the viscera to the muscles, thereby accelerating the metabolism of the latter (adrenalin is assumed to play a part) which

results in the production of excess lactic acid (due to more rapid oxidation).

That an excessively acid condition has long been associated with disease and may itself be a disease is evident from the term "acidosis." Acidosis should not be confused with acid stomach. The latter is not so serious and can be more readily treated, while acidosis (of the blood) is dangerous if chronic. The attitude of the medical profession toward it is not wholly uniform. While one physician will regard acidosis as "certainly not acid poisoning" and a decrease in blood alkali or hydrogen ions as merely incidental to something more fundamental, another will regard death from diabetes as due not directly to the diabetes but to the highly acid condition that results from faulty sugar metabolism. Untreated diabetic patients are on the acid side of neutrality, their pH being anywhere from normal to 7.00; acid accumulation is probably due to defective oxidation. Nephritis patients are also more acid, their pH occasionally being as low as 6.94. This represents an extreme minimum, as death is expected to occur at any pH below 7.

Van Slyke places the pH minimum of blood before which coma occurs at 6.95. This minimum has been observed in etherized dogs and in the blood of a nephritic man in coma a few hours before death. The other extreme lies at about pH 7.8, reached by voluntary deep breathing, causing the excessive giving off of carbon dioxide. Alcohol patients are on the alkaline side. A case of acute alcoholism may yield a pH of the blood of 7.61. The extreme range compatible with life thus lies between 7.0 and 7.8, and the normal range within 7.3 and 7.5.

Acidosis or alkalosis, whether or not it is a symptom or the cause of a disease, cannot always, and can never permanently, be remedied by the simple addition of an alkali or an acid—an error in medicine which has resulted in the use and abuse of sodium bicarbonate in therapy. The acid-alkaline equilibrium of the blood is adjusted by a very intricate regulatory system involving a delicate balance of carbonic acid, H_2CO_3 , and sodium bicarbonate, $NaHCO_3$. Giving the latter to an ill person may upset the proportion still further and make a bad matter worse. The feeding of alkalies, such as limewater to babies, is, therefore, now regarded by some as bad practice. One should, where possible, get at the cause. Acidosis in infants is due to deficiency

in a base, an alkali; this is, in turn, due to a lack of water, which causes urea to collect in the kidneys. Consequently, the proper treatment is to drink water. Acidosis in nephritis likewise causes kidney disorders; those organs cannot then secrete phosphate (KH_2PO_4).

It should not be assumed that a reaction against the medical practice of feeding alkalies to patients suffering from an acid condition means that the practice is altogether discarded or that it is not at all beneficial. In the case of diabetes, acidity may be so great as to lead to a state of coma. If, now, sodium bicarbonate is fed, there is a quick response toward the alkaline side which is to be detected immediately in the blood and urine. This gives instant relief from the acid condition which may help save the patient's life, but the relief is only temporary; the acid condition returns—the bicarbonate has not reached the cause.

Belief in the interdependence of health and pH outweighs skepticism. This is to be seen from the emphasis laid on the relation between health and acidity and alkalinity in foods. The pH of some common foods is given below (from La Motte Chemical Products Company).

Apples (or cider).....	2.9 to 3.5	Lemons.....	2.2 to 2.4
Asparagus.....	5.4 to 5.7	Milk, human.....	6.6 to 7.6
Beans.....	5.0 to 6.0	Milk, cows.....	6.4 to 6.8
Bread, white.....	5.0 to 6.0	Plums.....	2.8 to 3.0
Crackers.....	7.0 to 8.5	Squash.....	5.0 to 5.3
Corn.....	6.0 to 6.5	Tomatoes.....	4.1 to 4.4
Cherries.....	3.2 to 4.1	Water, sea.....	8.0 to 8.4
Dates.....	6.2 to 6.4	Water, distilled (in equilibrium with air).....	5.7 to 5.8
Grapes.....	3.5 to 4.5	Water, distilled (free from CO_2).....	6.8 to 7.0

Certain foods, acid or alkaline in themselves, may have "potential" alkalinity or acidity which is quite independent of the actual pH value in the natural state; that is to say, an acid food may have qualities that will lead to an alkaline reaction in the body. This property is possessed by lemons, which, while in themselves acid, produce an alkaline condition in the body. Citric acid, $\text{COOH}\cdot\text{CH}_2\cdot\text{C}(\text{OH})\cdot\text{COOH}\cdot\text{CH}_2\cdot\text{COOH}$, the chief acid of lemons, is tribasic; that is to say, there are three replaceable hydrogen atoms in the form of three carboxyl (COOH) groups. To the taste, this acid is sour; to the hydrogen-ion

indicator, it is a mild acid. In the stomach, through successive oxidations of the carboxyl groups, there are left alkaline residues (with OH⁻ radicals), so that what is sour or acid at first later becomes alkaline. We can express the facts this way. Lemons and oranges have an organic acid in them which tastes sour, is acid, and may cause an irritation of the stomach and therefore pain, but the final products of metabolism are carbon dioxide and an alkaline carbonate which is used to help maintain the correct balance between the acids and bases in the body. Also, citric (lemon) acid is a buffer; it may, therefore, function in this capacity in the stomach and reduce acidity present there. These two interpretations involve in a sense each other. Acids of this type are *metabolically alkaline*.

The foregoing agricultural and medical problems in acidity and alkalinity are of great importance in our everyday life. There are other pH problems which as yet are only of theoretical interest, as were all originally. Among these are the pH values of living protoplasm and of the sap of plant cells.

All organisms appear to possess gradients of a number of kinds, in metabolic activity, in salt concentration, in acidity, and in electric potential. Some of these gradients have been experimentally determined. A pH gradient implies that the body fluid of organisms is of different pH values at different points along the individual. This is not always easily proved, but an experimental indication of it can be given in a simple way by determining the pH values of the leaves and the basal stems of a plant. The leaves may have an average pH of 6.2; and the lower stems, 5.8.

In determining the pH of the living substance itself, the greatest care and ingenuity must be exercised. The earliest attempts at an acid-alkali determination of protoplasm involved merely the use of litmus paper, which indicated a pH of 6.8 for the protoplasm of a slime mold. Next came the experiment of Schaede, who immersed epidermal cells of the onion in methyl red and in a few minutes observed that the cell sap was red and the protoplasm yellow, which indicated an acid condition of the former and a basic condition of the latter. More recent experiments are those of Needham, Chambers, and others who have injected color indicators into the cell. The results give a pH of 7.6 for the protoplasm of Amoeba and 7.1 for other one-celled

animals. Eggs of echinoderms (sea urchin), a tunicate, and a worm are all about pH 6.6; yeast and other fungi (*Fusarium*) are between 5.9 and 6.1.

The protoplasm of the cells of higher plants appears to have a fairly constant pH value, being in this respect comparable to animal protoplasm (though there is some difference of opinion). The value generally accepted is 6.8 to 7.0. The pH of slime mold (*Physarum*) protoplasm, as indicated by its own natural indicator, a yellow pigment, is 6.2.

Extremes on the acid side of protoplasm may be great but not in view of the extraordinary behavior of certain plants. Some fungi (*Penicillium*) can grow on strong solutions of acid or on the underside of corks of bottles of acid. Extremes omitted, protoplasm is usually just on the acid side of neutrality—about pH 6.8. But Spek finds that there is not one pH value for protoplasm; in Amoeba, the thinner, fluid portion which advances at the tip of a pseudopodium has an acidity value of pH 7.3, while the inner (granular) protoplasm is 5.2.

Acidity determinations of plant sap, in particular the sap that fills the vacuoles of plant cells, are more readily made and are likely to be more accurate than those made of the cytoplasm. Dyes may be directly injected into the cell vacuole, the color change observed, and the pH value noted; or the cells may be bathed in dyes that penetrate easily. Often, the vacuoles contain pigments that are pH indicators. The colors of flowers, leaves, and fruits are due to pigments usually dissolved in the cell sap, *i.e.*, in the vacuole; less often is the pigment directly in the cytoplasm. Acidity values of plant sap range from pH 1.4 (G. N. Watson found this value for the India berry) to as high as pH 10. Values of the pH of cell sap are significant in themselves, but it is erroneous to regard a pH determination of the sap as indicating the acidity of the cytoplasm; the two may be of the same value, and undoubtedly the one influences the others, but the sap may be highly acid, and the cytoplasm near neutrality.

Attempts to measure the pH of the cell nucleus with dyes have been made, but the results are questionable, as a stained nucleus is usually a dead one. Results indicate a pH of 7.5 or 7.6 for the nucleus, as compared with 6.8 for the cytoplasm. The solution in which cells are bathed (blood plasma and tissue juices)

has a pH of about 7.4. The living cell thus appears to consist of an alkaline nucleus in acid cytoplasm, bathed in an alkaline medium.

The pH values so far given for protoplasm were obtained by the color method. These are all subject to the criticism of Deutsch (page 322) that in colloidal systems (sols and gels), owing to their great adsorptive surface, color changes take place which differ from those obtained in homogeneous solutions for which the color indicators were calibrated. (The error, however, may not always be very great. The pH values obtained for protoplasm appear, from every point of view, to be fairly accurate.)

Ingenious attempts to measure the pH of cells by the electrometric method have been made. Taylor and Whitaker have inserted, with the aid of a micromanipulator, delicate electrodes into the cells of the green alga *Nitella* and obtained the electrical potential and thus the pH of the cell sap. With a specially constructed microelectrode of hydrogen (a vessel holding but $\frac{1}{2}$ cc. of fluid), Bodine measured the yolk fluid from the *Fundulus* egg and found it to have a pH value of 6.39. With the same delicate electrode, he was able to determine the pH of insect blood of which but a drop is available.

The acid-alkali equilibrium of the living body is a very important and constant one, but it is not the only significant one. Calcium, potassium, phosphorus, and other elements play their part, both individually and in establishing a physiologically balanced solution; thus, the potassium ion is a very necessary one for plants, and the calcium-phosphorus balance is the determining one in bone formation. Still, we come to the conclusion that while in recent years overemphasis may have been laid on the hydrogen ion, it yet remains the most important ion in physiological reactions.

CHAPTER XVIII

ELECTROPHYSIOLOGY

Scientist, philosopher, and layman have long speculated on a possible relationship between life and electricity. It has been natural to do so, for life and electricity are forces of an extraordinary character. Increased knowledge has strengthened rather than weakened this old, half-scientific, half-superstitious belief in electricity as a potent and all-pervading force in life. To be sure, we are in an electric epoch and are likely to become overenthusiastic about electric forces, but there are many experiments to support electric interpretations of cellular behavior, and there is the fundamental fact that the ultimate unit of all matter is an electric charge. Relatively little is known of the precise part played by electric forces in organisms, but it has been possible to determine their presence there, to measure them, and to correlate them with certain physiological processes.

Magnetism and Electromagnetic Forces.—Electric forces may be dealt with under three headings—electromagnetics, electrostatics, and electrokinetics (these overlap more or less).

With pure magnetism we shall have little to do. It does not appear that organisms are sensitive to magnetic forces. The story is told that Faraday had a student place his head between the poles of a powerful electromagnet with no noticeable effect. However, some hypotheses have been advanced purporting to explain vital processes on the basis of magnetic forces.

So closely do the asters of a dividing animal egg (Fig. 17) resemble the distribution of iron filings within the field of influence of a magnet that magnetism was suspected of being the force responsible for the mitotic figure. This suggestion was made by H. Fol, when he first observed the mitotic figure in 1873. Magnetism would thus be the force under the influence of which the chromosomes migrate to the "poles" in a living cell. This speculation led to an attempt to disturb the normal process of cell division by placing a dividing animal egg in the field of a

powerful electromagnet. But nothing happened; the egg continued its process of division in quite the normal way. Still, hypotheses of the movement and orientation of chromosomes based on magnetic forces continue to be advanced. It has been found that magnets when floating on corks on water assume the same relative positions as do chromosomes.

Electrostatics.—Under electrostatics we shall consider but one phenomenon, *viz.*, the effect of static electricity on plant life. Experimental work of this nature has been done under the name of *electroculture*. That the air is electrically charged with respect to the earth, the difference in potential increasing with height, leads to the conclusion that plants out of doors with their roots in the earth and their tops in the air are constantly being traversed by minute currents of electricity. It is reasonable to assume that an increase or decrease in this current should affect the growth of the plant. From able investigators comes evidence interpreted by them as demonstrating that increased plant growth follows electric excitation of a certain intensity and duration, supplied from a charged network suspended above the plants. These investigations were begun by Oliver Lodge. Later, L. J. Briggs and other American workers took up the work and over a period of ten years treated plants in the field and in the greenhouse, under very carefully controlled conditions, with, throughout, negative results. There is a possible interpretation of the difference in results between American and English workers; if electricity is a substitute for light, an additional supply of it would, in England, be of advantage to the plants; but in America, where the average daily sunlight is in excess of the plants' needs, more energy in the form of a substitute would be of no benefit.

Work by Marinesco indicates that the beneficial effect of a static electric field may be due to an acceleration in the ascent of sap. The ascent of sap in plants is augmented by applying an electric field, provided the positive pole of the field is above the plant (Fig. 164). In order to obtain noticeable results, it is necessary to treat a plant in which the flow of sap is great, such as in tobacco.

Electric Currents.—Experimental physiology as we know it today began with the observation of Galvani that a muscle contracts if touched by two metal strips, one of copper and one of

zinc. From this he drew two conclusions—that the production of an electric current is a cause of muscular contraction and that the origin of the current is due to a life force within the muscle. The first deduction is correct; the second, not justified.

That a current of electricity will produce muscular contraction is now a well-known fact. It is also true that electric currents arise in muscles but not in the way in which Galvani thought. What actually took place in Galvani's experiment was the establishing of the essentials of a typical galvanic cell. Strips of copper and zinc in an electrolytic solution produce a current. Galvani supplied the two metals, and the muscle possessed sufficient salt and water to play the role of an electrolytic solution. A current was produced, and the muscle contracted.

This physiological experiment of Galvani's, performed in Bologna in 1789, led Volta of Pavia, in 1800, to put together the kind of electric cell that we now know by his name. The experiment of Galvani on muscle contraction was the first in electrophysiology and among the first in dynamic, or *galvanic*, electricity.

The new science of electrophysiology was not put on a substantial basis until fifty years after Galvani, when the German physiologist duBois-Reymond established the fact, which Galvani thought true even though his experiment did not prove it, that organic tissues give rise to electric currents. DuBois-Reymond made the classical observation that if tissue is wounded, there is immediately set up an electric current which flows from the wounded to the nonwounded region. The nature of the wound is immaterial. But it is not necessary to wound an organism in order to produce a current. Tissues constantly maintain differences in potential between each other. Tendon is always negative to muscle, and root negative to leaf or stem. There thus results a flow of current (*i.e.*, of electrons) from tendon to muscle and from root to leaf if the two tissues are connected by a conductor. Within the body, the salt solutions of the tissues may possibly serve as a conductor (pages 344-345). That electric forces are involved not only in muscular action but in many if not all physiological processes seems very likely. Michaud has shown that merely touching a gel causes a difference in electric potential, which can be measured. If this is true of nonliving gels, it should be true of living gels, such as muscle, nerve, and protoplasm in general.

Nerve Conduction.—Recent studies on nervous impulses show a very definite relationship between electric and vital reactions. Early speculations on nerve action assigned to the nerve fiber a purely passive role. It was supposed to carry a stimulus from one end to the other, as would a wire or pipe. Later, the discovery was made that an electric potential is set up by the nerve itself during activity, the intensity of the charge being independent of the nature of the stimulus applied. This indicates that electricity is associated with the transmission of an impulse in nerves, but it does not distinguish between cause and effect.



FIG. 149.—The circuit of an excitation wave along a nerve fiber. The shaded portion is the momentarily activated region (its length is 6 cm.); the maximum potential reached is 40 millivolts.

Among the first hypotheses of nerve conduction was that of Ralph Lillie, who postulated that there is an essential similarity between a nerve impulse and the conductance of an electric current by the surface film of certain metals. The reaction of iron wire and nitric acid exhibits an automatic rhythm of often remarkable regularity, consisting of an alternation of active and passive periods. Waves of activation travel along both wire and nerve at intervals followed by passive, or nonreactive, periods (Fig. 149). The wave impulse sets up an electric eddy, or a succession of such eddies, as it travels forward. The number of cycles per minute in iron wire varies between 40 and 100 or more, and is determined by several factors including the constitution of the wire, the concentration of the acid, and temperature. A wire freely suspended is nonrhythmic. Contact with glass or another surface appears to be necessary. The wave is apparently one of oxidation and reduction of the iron on the *surface* of the wire (see page 341). It does not take place on copper wire, and it is not a current comparable to a flow of electricity. There is no constant fall of potential along the wire as in an electric circuit, but instead a succession of waves or impulses of chemical reactions and consequent electric potentials. This leaves the wire positively charged over a short section when oxidation is taking place and negatively charged when reduction is going on. In time, the wire spontaneously ceases activity, the potential drops,

irregular fluctuations in activation occur, until a level of complete passivity is reached. All these reactions are duplicated in living nerve tissue. Lillie points out that the dissimilarity in the chemical constitution of iron wire and nerve fiber in no way contradicts the identity of the fundamental physical reactions in the two systems.

The work of Ralph Lillie established a foundation for subsequent investigations. Other suggestions were made, such as local movements of ions by diffusion, but this would be too slow to account for the very rapid transmission of nerve stimuli. A flow of electrons in the sense of a true electric current has also been suggested. Emphasis on the chemical nature of nerve transmission is based on the fact that a nerve slowly loses its conductivity under conditions of asphyxia and recovers with oxygen, which suggests a chemical reaction (oxidation reduction) as the process responsible for the activity, but we have seen that this is also the basis of Lillie's hypothesis (there is no essential difference here between what is chemical and what is physical). Physiologists are now generally agreed that the nerve stimulus is an electric disturbance, wavelike in character. The velocity of the waves has been established to be between 30 and 90 m. per second, and their length about 18 mm. Erlanger, Bishop, and Gasser have photographed the wave.

Two other workers, E. D. Adrian and D. W. Bronk, have carried studies of nerve transmission to a very refined point, especially in regard to technique. Adrian supports the conclusions of Lillie, that the activity of neurons or nerve cells and fibers is rhythmic owing to a rapid breakdown and repair of their surface films. The larger nerve fibers or trunks are like cables in that they are bundles of many finer fibers each of which is capable of carrying an independent message. There may be a thousand such fibers in one fair-sized nerve. It has been the contribution of Bronk that a single fiber can be dissected apart from the others and left intact, and its message alone studied and recorded (Fig. 150). The extraordinary conclusion is reached that the kinds of nerve impulses (whether sight, hearing, touch) are alike in magnitude, rate of travel, etc. The frequency of the nerve impulses depends on the intensity of the stimulus; they vary from 300 to as low as 10 per second. How, then, is one kind of message distinguished from another? This is done by

the central nervous system, where the impulses are received. The work of the English physiologist Sherrington and of the Russian Pavlov has had to do with this question of the nature of the impulses sent out by the central nervous system in relation to the kinds received by it. Foerster has demonstrated that stimulation of the temporal lobe of the human cortex may cause sounds and words to arise in consciousness and that stimulation of the occipital lobe gives light or images.

It has been possible to record with remarkable mechanical precision the rhythmic nature of nerve impulses and to gain some knowledge of the chemistry and physics of these impulses.

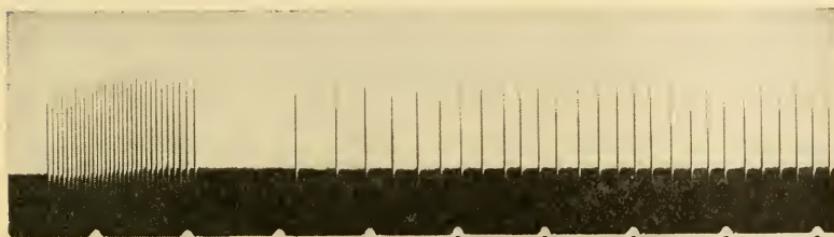


FIG. 150.—Record of a train of impulses or action potentials of single nerve fibers.
(From D. W. Bronk and H. K. Hartline.)

Important as are these studies, they still leave us ignorant of the mechanism of the nervous system as a whole, of learning and memory, though these latter have been interpreted by the psychologist K. S. Lashley in terms of a mechanism involving the activity of groups of neurons or cerebral centers.

Electrolytic Conduction.—The passage of a current through a solution is known as *electrolytic*, or *ionic*, conduction in distinction from *metallic*, or *electronic*, conduction in a wire.

Conductance is the reciprocal of resistance; *i.e.*, conductance = $1/\text{resistance}$. We may, consequently, determine the conductivity of a wire or a solution by measuring the resistance. Resistance is measured electrically by the setup known as Wheatstone's bridge.

In determining the resistance of an electrolytic solution, a direct current cannot be used, because it brings about electrolysis, and readings after one, two, or three minutes will differ. An alternating current must be employed. It is usually supplied by inserting a small induction coil which receives its current from a battery. The use of an alternating current precludes

using the customary type of galvanometer, which is, therefore, replaced by a telephone receiver; minimum buzzing of the latter indicates the point on the wire (the "null" point) where equilibrium is reached. A conductivity cell or vessel having platinum electrodes which are immersed in the volume of liquid to be measured is used. Its *cell constant*, or *capacity factor*, must be determined. This factor takes account of all physical properties peculiar to the vessel, such as the size of and distance between the platinum electrodes.

Electrolytic conductance measurements serve as a means of determining the salt concentrations of soil water, the sea, and the body fluids of plants and animals. Obviously, the amount of nonelectrolytes (sugars, etc.) present is not indicated by conductivity determinations. Osmotic pressure is an index of the combined concentrations of electrolytes and nonelectrolytes in solutions. The conductivity of plant juices (sap) has been used as an indicator of the amount of electrolytes that have entered or left the tissue. It is thus an indicator of changes in the permeability of the living cell. The green alga Valonia encloses a large vacuole or sac which can be tapped and the sap within collected. The amount of sap obtained is rather small for convenient quantitative chemical analysis but sufficient for quick conductivity determinations which indicate the total electrolytic concentration. Juices may be extracted from plant tissues by pressure, and values of total concentration of electrolytes determined by conductivity measurements. The juice from an onion extracted by pressure has a conductivity of 6×10^{-3} mhos, which is equivalent to about 0.05*N* potassium chloride.

Conductivity has been used to indicate the electrolytic concentration of solutions (water cultures) in which plants are experimentally grown. In this convenient way, the amount of salts taken out of solution or the salts given off by plants when grown in distilled water can be determined.

Lecomte du Noüy has determined the conductivity of horse blood and found it to be $0.012168 = 12 \times 10^{-3}$ (mhos), which is just twice that of the plant juice given above.

A conductivity value of protoplasm has been obtained by S. C. Brooks, who found it to have a resistance of 19,000 ohms, which represents a very low conductance, equivalent to 0.00145 *M* sodium chloride. Gelfan found a higher conductance for the

protoplasm of the starfish egg, a value equivalent to a 0.25 M potassium chloride solution.

Potentials in General.—There are a number of types of electric potential which are definite in character, in that we know exactly to what they are due and under what conditions they may be expected to arise. This is true of the potentials ordinarily studied by the physicist, such as that between a metal and its surrounding solution, which is evaluated by determining the difference in potential between two such electrodes. Equally definite is the potential existing between the outlet and intake of a dynamo. In colloidal and biological systems, the situation is not so simple. Most potentials there are due to a combination of electric forces.

Eucken and his translators Jette and La Mer have given a satisfactory treatment of potentials from the physicochemical point of view, but it is incomplete from the colloidal and biological viewpoints. The following is a modified and augmented classification:

Electrode potential, metal/solution.

Concentration potential, solution/solution.

Diffusion potential, liquid/liquid (miscible).

Liquid junction potential, liquid/liquid (immiscible).

Oxidation-reduction potential, oxidizing system/reducing system.

Membrane potential, solution/membrane/solution.

Injury potential, injured tissue/normal tissue.

Electrode Potentials.—The potential difference at the interface of a metal in contact with a solution was first mathematically expressed by Nernst (1889). The assumption is made that the metal goes very slightly into solution. It gives off ions which are held at its surface (in the interface) so that a double layer of ions is formed, with a resulting charge on the electrode (Fig. 151).

Such an electrode potential as the preceding exists on the two poles of an electric cell, *e.g.*, the copper and zinc electrodes of a galvanic cell. Between these electrodes there is a potential difference which is designated as the *electromotive force* (e.m.f.) of the cell. Anions migrate in

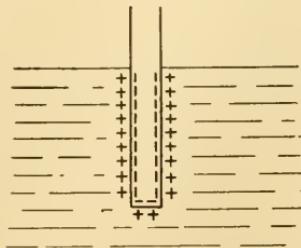


FIG. 151.—The double layer of ions formed at the surface of a metal electrode within an electrolytic solution.

one direction and carry with them a definite quantity of negative electricity; and cations migrate in the other direction, carrying positive electricity.

Electrode potentials as such do not enter into living systems normally, but, owing to polarization, they are often bothersome when measurements of other potentials are being made with the aid of metal electrodes. For this reason, metal electrodes are not satisfactory for measuring potentials in living tissues. Special contacts or junctions (*e.g.*, of agar saturated with salt) have to be resorted to (Fig. 42).

Contact and polarization potentials are potentials not unlike electrode potentials but with other names. When a current is passed through a circuit, the source of electromotive force then removed, and the circuit closed again, a current may begin to flow in the opposite direction, as in an accumulator. This back current presupposes a polarization potential.

Concentration Potentials.—The electrodes of a galvanic cell are of two different metals in a common solution. If electrodes of the same metal are put into separate solutions of the same salt but of different concentrations, and the two electrodes joined by a conductor, a flow of current will result due to a potential difference between the electrodes. Such a potential is known as a *concentration potential*, and the cell as a *concentration cell*.

Diffusion Potentials.—A diffusion potential, sometimes referred to as a liquid junction potential, is produced at the surface of contact between two miscible solutions. It is due, as Nernst first pointed out, to the unequal diffusion of ions across the boundary from one to the other liquid due to the different migration velocities of the ions. Some migration numbers are given in the following table:

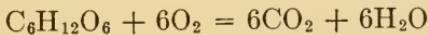
Ion	Absolute velocity, cm./sec. $\times 10^{-4}$ at 18°C.	Ion	Absolute velocity, cm./sec. $\times 10^{-4}$ at 18°C.
H ⁺	32.50	OH ⁻	17.80
K ⁺	6.78	Cl ⁻	6.78
Na ⁺	4.51	NO ⁻	6.40

The hydrogen ion moves at 0.00325 cm. a second, and the nitrate ion at 0.00064 cm. a second. The fact that the faster

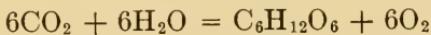
ions, such as H^+ , are soon in advance of the others results in an unequal distribution of ions and therefore of electric charges, which produces a potential gradient at or near the diffusion front where the faster ions are in the lead. Except for the rapid H^+ ion and the moderately rapid OH^- ion, all potentials due to differences in velocity of ions are not great and will, in any case, be quickly obliterated through equalization in concentration when the slower ions catch up; furthermore, one ionic species cannot separate far from the other without setting up a very great potential difference. The electric force thus produced tends to slow up the faster ions and hasten somewhat the slower ones. However, if the fluid boundary is maintained by a constant flow of the two solutions against one other, a measurable potential is established.

Liquid Junction Potentials.—A liquid junction potential is set up at the interface between two immiscible liquids. Such an interface maintains itself permanently. The potential results not because of different rates of diffusion of ions across the boundary, as in the case of diffusion potentials, but because of different degrees of solubility of ions in the two phases. For example, water and amyl alcohol are immiscible, and H^+ and Cl^- ions are not equally soluble in them. If they are shaken with hydrochloric acid, the two liquids (water and amyl alcohol) separate and form a sharp boundary where a potential is established owing to the unequal solubility of the H^+ and Cl^- ions.

Oxidation-reduction Potentials.—Priestley, in 1774, discovered that a particular constituent of air, which he called "dephlogisten," is necessary to life; Lavoisier called this substance oxygen. Since then, the importance of oxygen in physiological reactions has become increasingly evident. The prime use to which oxygen is put by organisms is the oxidation of foods, among which the sugars have been most studied, though fat and protein oxidation is quite as important. For sugar, the equation is



When sugar is formed by plants through photosynthesis, oxygen is liberated:



Because this latter process involves the release of oxygen, it is known as *reduction*, which is defined as the reverse of *oxidation*.

It is often stated that such and such a process is oxidation, and another reduction; thus, respiration is said to be oxidation, and assimilation reduction, but actually the oxidation of one substance always involves the reduction of another. In the first of the two preceding formulas, the sugar is oxidized and the oxygen reduced; and in the second, the carbon is reduced and the oxygen oxidized. If oxidation and reduction processes are analyzed more carefully, it is seen that what actually happens is an increase in positive valence with oxidation and a decrease in positive valence (or increase in negative valence) with reduction (this is to be understood as involving only a change in the number of valence electrons; reduction involves an increase, and oxidation a decrease of electrons); thus:

Valence	Oxide of N	Hydroxide or acid
+5	N ₂ O ₅	HNO ₃
+4	N ₂ O ₄	
+3	N ₂ O ₃	HNO ₂
+2	N ₂ O ₂	
+1	N ₂ O	HNO
0	N ₂	
-1	NH	NH ₂ OH
-2	N ₂ H ₄	
-3	NH ₃	NH ₄ OH

When iron in the ferric condition (*e.g.*, as FeCl₃) is reduced to the ferrous condition (FeCl₂), the metal gains a negative charge (a decrease in the number of positive charges obviously corresponds to a net increase in negative ones); thus:



As the ion Fe⁺⁺ is capable of losing an electron, it is regarded as a reducing agent, or *reductant*; and as Fe⁺⁺⁺ is capable of gaining an electron, it is an oxidizing agent, or *oxidant*. The change from one to the other is a reversible reaction and may be expressed thus:



An important distinction lies between the oxidation of Fe⁺⁺ and the oxidation of sugar in metabolism; the former is reversible,

and the latter not. By a perfectly reversible chemical reaction is meant one that requires the application of the same amount of energy to reverse it as that yielded by the original reaction. Oxidation-reduction systems may be completely reversible, partially reversible, or irreversible, with the fact kept in mind that a system that under one set of conditions is completely reversible may under another be partially reversible or irreversible. There is no conclusive evidence that any system is irreversible under all conditions.

The reductant Fe^{++} has one more (negative) electron than the oxidant Fe^{+++} ; an electron was transferred from the oxidant to the reductant. This fact has led to careless reference to potential and flow of current in living systems. In the first place, there is no convincing evidence that electrons can occur free in aqueous solution for any appreciable length of time. Metal conductors carry a flow of electrons, but aqueous solutions require discrete carriers of electrons (ions) for the conduction of electricity. It appears, moreover, that there does not exist in living systems any mechanism that is analogous to the metallic electron conductor. (We shall question this statement in a moment.) This being true, then any theory of electric potentials in tissues must be based upon a different viewpoint from that which holds for the outer circuit of a galvanic cell. We know that living systems are demonstrably chemical systems in which material of high chemical potential is degraded to material of low chemical potential. This means that one may calculate a theoretical electron pressure difference corresponding to the difference in chemical potentials. An electron pressure difference is an electric potential difference. But we have made one assumption, *viz.*, that the chemical energy, concerning the reality of which there is no doubt, is converted into electrical energy in the living system. Some of it is so converted when we pick it up and measure it by means of metal electrodes, but this is not evidence that the energy liberated in the living system is in the form of electric energy. We may restate this thought as follows: In spontaneous chemical reactions, there is a decrease in the level of free energy. The energy thus liberated is available for work, which may be mechanical, chemical, electrical, thermal, etc., depending on the paths that happen to be available. The paths represent mechanisms, and we are at liberty to postulate *any*

mechanism for purposes of thermodynamic *formulation*, provided that it does not violate the fundamental postulates of thermodynamic theory. Our postulates give no clue as to which of the mechanisms is the actual one. Because we utilize some of the free energy by means of a platinum electrode and thus produce an electric current that can be measured, this does not imply that an electric current is produced in the system. In other words, we measure and express the level of free energy in terms of an electric potential simply because the potentiometric method is usually the most convenient one for measuring the difference in energy level. We could just as well express the free energy in terms of a different scale—say, for instance, in terms of calories.

Although the method of measurement, rather than the system itself, may give rise to electric potentials, yet, as there is a decrease in the level of free energy, conduction by means other than direct electron conduction may take place, *viz.*, by electrolytic conduction. The experiments of R. Lillie (page 335) give further support to the possibility that an actual flow of current takes place in living systems. There is a great similarity between the flow of current along (the surface of) an iron wire immersed in an electrolytic solution and the conduction of an impulse along a nerve. As this similarity is very pronounced, there may yet be something within the nerve that is analogous to the metal. Lillie has himself considered this possibility and appears to be of the opinion just stated, *viz.*, that there are regions in living tissues at which oxidation and reduction occur in dependence on a flow of current and therefore on metallic or like conductors. In the comparison with the condition in passive iron, it is assumed by Lillie that the oxide film behaves in a way similar, in a general electrochemical sense, to that of the plasma membrane of the irritable living element. Oxidation occurs at one region simultaneously with reduction at another region at some distance, and the associated structural changes involve changes of potential of such a kind that the effect is automatically transmitted. We do not know if there is anything in the nerve to correspond to a metal electrode or conductor, but the essential factors are the same in the iron and in the nerve.

Chemical reactions occur on the passing of a current across a membrane in contact with solutions containing reactive com-

pounds on opposite sides of the membrane (oxidation-reduction reactions at membrane surfaces are known as *electrostenolysis*). In such cases, where electrons are lost from oxidized molecules and in some way an equivalent number of electrons reach the reduced molecules on the other face of the membrane, there must, it would seem, be a combination of electrolytic and electronic conduction. Lillie has suggested that carbon chains may serve as electronic conductors in living systems.

If we conclude that potential is a measure of the level of free energy and that the voltage registered by a potentiometer measures simply a *tendency* for electrons to go from one place to another (*i.e.*, voltage is the *intensity* factor of energy), we do not thereby deny the presence of those phenomena in living systems which involve an actual transfer of electrons during the course of an oxidation-reduction reaction. This is the case of nerve conduction (page 335), which is a wave or series of waves of oxidation and reduction. Here there is a dynamic process involving chemical reactions in which electrons may be moving. The chemical reaction requires a flow of electrons in order that it may take place; the potentiometer measures the tendency to flow.

A mixture of ferrous and ferric chloride in an electrode vessel will yield an oxidation-reduction potential. It is usually expressed in volts with reference to the normal hydrogen electrode, the potential of the latter being taken as zero. Two factors primarily determine the magnitude of oxidation-reduction potentials—the ratio of oxidant to reductant and (in most organic systems) the activity of the hydrogen ion.

Oxidation-reduction potentials are of the order of 1 volt, varying from less than the +0.2 volt of the system $\text{Cu}^+/\text{Cu}^{++}$ to more than the +1.8 volts of the system $\text{Pb}^{++}/\text{Pb}^{++++}$. (Temperature is a factor.)

Oxidation-reduction Potentials in Living Systems.—The measuring of the oxidation-reduction potential of living cells usually consists in bathing the cells in, or injecting into cells, color indicators covering the scale of potential values from that of the hydrogen electrode to that of the oxygen electrode. The dyes are taken in sequence, and their reactions observed in the cell. A dye, such as methylene blue, will be oxidized (and retain its color) or reduced (and lose its color) depending upon

the relative activity of these two processes in the cell. With a definite, limited series of indicators, a point on the scale can be found that will indicate the potential (if the system is in equilibrium) or, if not that, then at least the relative rates of oxidation and reduction.

Thus do dyes indicate the reduction potential of tissues by color changes analogous to those which indicate pH values. (The electrometric method has so far proved impossible for studies on cells.) In some cases the cells themselves may contain their own naturally occurring reduction indicator, such as echinochrome in Echinoderm eggs, and cytochrome in yeast and other cells.

Oxidation-reduction potentials were formerly expressed in terms of rH, a symbol devised by W. M. Clark to express similarity between oxidation-reduction potentials and potentials set up by hydrogen ions. The analogy with pH was shown by defining rH as $\log \frac{1}{\text{H}_2 \text{ pressure}}$. An oxidation-reduction potential of 0.81 would have an rH of 41; and a potential of 0.072, an rH of 11.6. As the analogy is not complete, the symbol has been dropped.

Apparently, Gillespie was the first to measure an oxidation-reduction potential produced by organisms (cultures of *Bacterium coli*). W. M. Clark began his extensive studies with an investigation of the reducing power of milk. Needham was the first to apply the microinjection method to studies on oxidation, which have been extended by Rapkine and Wurmser and particularly by Cohen and Chambers. M. M. Brooks employed the immersion method by bathing cells in dyes which enter readily; the cell sap of the alga *Valonia* was found to have a potential of +0.12 to +0.15 volt (an rH of 16 to 18); and the protoplasm, a potential of 0.21 to 0.48 volt. Many potential studies have been made of bacteria and yeast, though obviously not of the interior of the cell itself but merely of the surrounding solution.

It is, therefore, necessary to differentiate between potentials obtained within cells and those obtained in cell suspensions. Potentials in cell suspensions must be referred to cellular products which have passed into the suspension medium. Values for cell suspensions have, as a rule, been obtained by the electro-

metric method, *i.e.*, by direct electrical measurement, and not through color indicators.

A significant result of oxidation-reduction potential measurements on both cells and cell suspensions is that more negative potentials are found under anaerobic as contrasted with aerobic conditions, demonstrating that the "reducing intensity of the cell" is not poised at a definite level. Cohen suggested the term "reducing intensity" instead of "oxidation-reduction potential," to meet the objections that there is no electric potential as such in tissues and that thermodynamic equilibrium does not occur. He and Chambers found the reducing intensity of the aerobic cell (*e.g.*, Amoeba) to be -0.07 volt (assuming the pH to be 7).

In addition to knowing whether the values obtained represent true potentials or merely relative rates and whether or not they are of thermodynamic significance, there is the almost insuperable difficulty, as D. E. Green and Szent-Györgyi say, of relating the observed potential to the multitudinous factors that determine it. These are the heterogeneity of the cell and the multiplicity of reversible, semireversible, and irreversible oxidation-reduction systems present; the discrepancies obtained in the values themselves when indicators differing widely in chemical nature are employed, owing to the fact that the state of oxidation-reduction of an indicator is determined not only by factors in the cell but also by the toxicity, poison action (buffering), autoxidizability, and kinetic behavior of the indicator; and the ease with which the values may be shifted by narcotics, penetrating acids and bases, and changes in the oxygen tension.

Perhaps the most promising field for further investigation in oxidation-reduction potentials is the isolation and study of oxidation-reduction systems occurring in cells and organisms.

Various oxidizing enzymes with their substrates, which do not themselves affect electrodes, have been shown to establish oxidation-reduction potentials which can be detected by using reversible dyes. The cytochrome of yeast and other cells is itself a reversible oxidation-reduction indicator, as also are the recently discovered flavines. Various reducing substances found in living tissues, such as ascorbic acid (vitamin C), glutathione, and other sulphhydryl compounds, have probably a considerable influence in determining the oxidation-reduction

potential of cells. Wurmser also refers to certain derivatives of sugar.

In conclusion, it seems that the most that can be said of the work done on oxidation-reduction potentials in living systems is that the potential value obtained is at least an indication of relative metabolic activity.

Membrane Potentials.—Membranes, such as parchment paper, collodion, skin (of plant or animal), the lining of intestines and the protoplasmic membrane of cells, have the extraordinary property of allowing only certain ions to pass through or certain

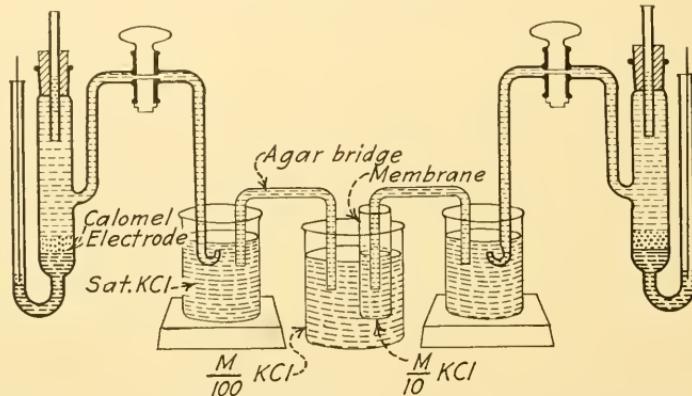


FIG. 152.—Chain of connections for measuring the difference in potential between the two sides of a membrane.

ions to pass through more rapidly than others. Such membranes are said to be differentially, selectively, or semipermeable. Most nonliving and all living membranes are selectively permeable. Thus, parchment paper and collodion membranes permit positive ions to pass through more readily than negative ones, and the apple skin is likewise more freely permeable to the cation potassium than to the anion chlorine. If a selectively permeable membrane separates electrolytic solutions, there will result a different distribution of the ions on the two sides of the membrane from that which existed at the beginning of the experiment. A membrane potential results which may be measured with the aid of electrodes and a potentiometer (Fig. 152). The values are usually of the order of 50 mv. (In Fig. 152, two calomel electrodes are shown. Here they do not serve as reference electrodes, as in pH measurements, but are used in going from metallic to liquid conduction so that they may

exactly balance each other. One always checks them against each other to see that they really cancel out and that the current flow is zero. If they thus cancel each other, then any potential observed in the system can be safely referred to some other site than the metal-electrolyte junctions. Junction potentials are minimized by the use of saturated potassium chloride cells or bridges or both. The mobility of the potassium and chlorine ions is so nearly identical that no appreciable electromotive force arises at such junctions. There remain only the electrical stresses across the membrane to produce an electromotive force which is balanced by the potentiometer.)

The potential difference between the two sides of anatomical membranes involves not only the electrokinetics of the unequal distribution of ions but also the mechanics of the membrane. Numerous hypotheses have been advanced (see pages 275, 290). Here we shall mention but one of them—that which ascribes the selective ionic permeability of membranes to an intrinsic electric charge.

If a membrane—to be specific, an apple skin—possesses a slight initial charge which is negative, then anions, such as Cl^- , will be repelled by the membrane and cannot pass through, while cations, such as K^+ , will not be repelled and can pass through. The apple skin should therefore be more permeable to cations than to anions, as Michaelis found to be true. This unequal distribution of ions across the membrane establishes a potential which increases the initial charge of the membrane. It is this final potential that we measure.

Wilhelm Ostwald suggested that the electric forces in muscles and nerves have their origin in the selective permeability of living membranes, in that the membranes are permeable only to anions or cations. The theory has proved to be a very useful and significant one, in that it places the seat of vital electromotive forces at phase boundaries or membranes. Donnan interpreted the theory of Ostwald mathematically. Equilibrium (page 203) brings about an unequal distribution of ions which results in a potential difference between the two sides of a membrane.

We may classify membrane potentials, in the main, into two groups—those resulting from ionic (a Donnan) equilibrium and those resulting from the unequal diffusion of ions through the

membrane. The former may be permanent, but the latter must be constantly changing in order to maintain itself and is therefore a likely condition in living systems.

The migration of ions, whether freely in a solution, selectively through a membrane, or under the influence of an electric field, involves the question, How far can an ion get away from its mate? It has already been stated that one ionic species cannot separate far from the other without setting up a very great potential difference. In a voltaic cell, or in electrolysis wherever it occurs, positive ions move in one direction, and negative ions in the opposite direction. They probably do not travel freely, *i.e.*, wholly alone, but pass from one ion to another. They thus leave their original mates and pass on to others. In saying, therefore, that a membrane is selectively permeable to cations—that the apple skin permits potassium to pass through more readily than chlorine—it is not intended to imply that there is a true and complete separation of the ions without other ions taking the place of the discarded mates. There is an *interchange* of ions between the two sides of the membrane. Electric neutrality (equilibrium) must be maintained in static systems and striven for in dynamic (living) systems. This difficulty may be avoided by assuming that there is no abrupt change in the potential at the surface of the membrane but a potential gradient from one side to the other. The membrane does not sharply separate two ionic species and therefore two surfaces of different potential; there is, instead, a gradual transition between the potentials of the two sides.

The role of membrane potentials in vital phenomena is probably a very great one, because there are many membranes in living systems, and each is the seat of an electric potential. All living membranes, whether the covering of tissues, of cells as a whole, or of cell parts, are differentially permeable and are bathed in electrolytes. This is sufficient to indicate that living cells are generators of electromotive forces. Theoretically, this must be true, but there is experimental evidence as well. The apple skin is more freely permeable to cations than to anions. Potassium passes through more readily than chlorine. As a result, the tissue within the apple is left with a negative charge, and the outer solution becomes positive. That a potential is actually established can be proved by measurement, as has been done by

Loeb and Beutner. The complete apparatus for performing the experiment is illustrated in Fig. 153, and the diagram given in Fig. 154.

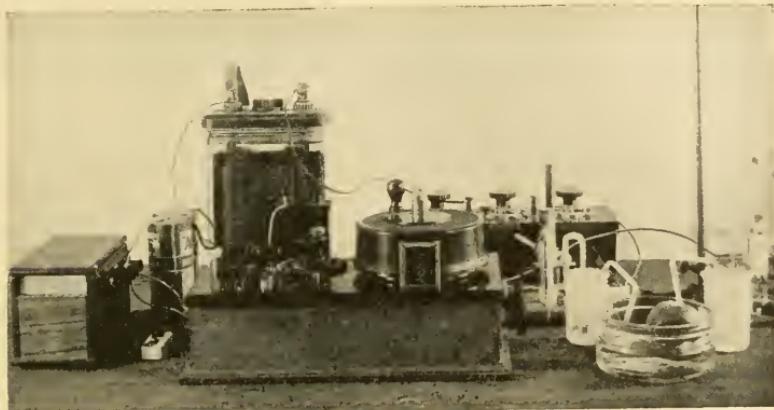


FIG. 153.—Apparatus for determining the potential difference between the cut and the whole surface of an apple.

An apple is put in a bowl containing a salt solution ($0.01M\text{KCl}$). A depression cut in the top of the apple holds a similar salt solution and an electrode (or agar bridge leading to an electrode). The apple, with its solutions and electrodes, is inserted as the unknown electromotive force in a potentiometric circuit, just as in the case of an electrometric setup for determining pH (Fig. 145). If an external electromotive force (storage cell) is first balanced to a known potential, *viz.*, that of a standard

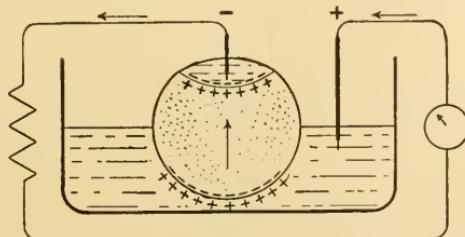


FIG. 154.—Diagram of the flow of current (*i.e.*, of electrons) from the cut (*upper*) surface of an apple containing a salt solution and an electrode, through a resistance, a galvanometer, an electrode, a surrounding electrolytic solution, to the whole (*bottom*) surface of the apple and through the apple.

cell, and then to an unknown one, *viz.*, that of the apple skin, the unknown potential can be calculated (page 314). A potential of some 40 mv. (0.040 volt) is thus obtained for the apple.

This is the difference in potential between the upper cut and the lower intact surfaces. The region between, within the apple, is a rather complicated system consisting of the upper wounded surface, the lower skin surface, and the intervening pulp with electrolytes. The potential measured is, consequently, no simple or single one. It involves at least three potential differences, *viz.*, that between the inner and outer sides of the cut surface, that between the inner and outer sides of the intact skin, and that between the cut surface and the intact skin. The direction of flow of current (*i.e.*, of electrons) is from the injured region at the top of the apple, through the surrounding solution, the intact apple skin at the bottom, and finally through the pulp of the apple. The upper, cut surface of the apple is, therefore, negative; and the lower surface of the skin, positive. Within the apple, the flow of current is from the inner surface of the lower untouched skin, which must, therefore, be negative, to the inner surface of the upper, cut area, which is, therefore, positive (Fig. 154).

Membrane potentials are generally obtained when other tissues, such as frog skin or leaves, are bathed in salt solutions.

R. Höber undertook an extensive series of experiments in an effort to ascertain which ions living membranes permit to pass and which not and, therefore, which ions are responsible for membrane potentials. The responsibility cannot be wholly attributed to this or that ion, but it does appear that the unequal permeability of the cell membrane for potassium and chlorine ions is a large factor in determining membrane potentials in vital systems.

Injury Potentials.—There remain a number of potentials capable of measurement and therefore, in this sense, real, which the physicist will regard as involving other factors in addition to those to which they are ascribed. One such potential is that due to injury. The actual physical basis of an injury potential is unknown; it is due probably to an upset in ionic equilibrium caused by the injury. Whatever the ultimate cause may be, the potential is the direct result of injury and is capable of measurement. It is not a simple potential but involves others. It does, however, manifest itself clearly and was one of the first forms of vital potentials discovered. If tissue is injured, there is a flow from the injured to the uninjured region. The potential,

when measured immediately after injury, is higher than that measured later. The latter is a normal membrane or concentration potential or both. The difference between the total potential first measured and the final potential is that due to injury.

The increased acidity of injured regions is a possible cause of injury potentials. Increase in hydrogen-ion concentration indicates electric activity. Furthermore, freshly exposed surfaces of tissues oxidize readily. The oxidation itself, as well as the greater acidity of the tissue, indicates that injured tissues are the seat of electric forces.

Other Potentials.—Potentials exist wherever there is an interface separating ions of different distribution. We can, therefore, increase considerably the list of potentials—if not of actual ones, then merely so-called ones—which are useful as names to designate specific instances. Such are surface potentials, at the interface air-liquid (a fairly well recognized potential); and, if we select the particular ion responsible, then, hydrogen-ion potentials, between acid-conductor-base (a kind of concentration potential). There are also electrokinetic potentials due to selective adsorption and ionization (of proteins); but, as they do not involve a flow of current and cannot therefore be measured directly, they will not be considered here.

It will be noticed that in this discussion of potentials no extensive use has been made of mathematical formulation. This is unnecessary for the operational definition and classification of phenomena.

The role of all types of potentials in life is not fully understood other than that they release energy. As life is the sum total of all energy manifestations that occur in protoplasm, electric potentials are a part of that whole which we call life.

Potential Differences between Cell Parts.—Potential differences between body parts—for example between injured and uninjured tissues—are capable of experimental proof. In the case of the individual living cell, there is no satisfactory way of measuring potential differences; but evidence that they exist, though indirect, is nevertheless to be had. There is, first, the fact that any system containing a selectively permeable membrane bathed in electrolytes is certain to have potential differences, due to an unequal distribution of ions. Such potentials must exist within living cells and between one cell and another. The

further fact that different regions in a cell are more or less independently engaged in physiological activities suggests that their ionic activities differ, which is but to say that their electric activities differ and that there are therefore potential differences between them. Proof of differences in acidity is sufficient evidence that differences in electric pressure exist. That this is true is evident from the fact that one of the ways of measuring the hydrogen-ion concentration of a system is to measure the potential set up by the ions. The same is true of other activities in the cell; thus, if the rate of oxidation differs in various parts of the cell, a potential is set up. Indeed, any type of chemical activity involving the liberation or interchange of ions will produce a potential. We need only, therefore, establish evidence that differences in such activities exist within the cell in order to have proof that the cell is a source of electric forces.

Basic dyes, which ionize as R^+ and Cl^- , stain the nucleus more readily than they do the cytoplasm. The nucleus is, therefore, electronegative, because it adsorbs the positive or color ion R^+ of the dye. Cytoplasm, on the other hand, stains better with acid dyes which ionize as H^+ and R^- . It is, therefore, less negative than the nucleus, *i.e.*, electropositive in reference to the nucleus. Similar evidence leads to the conclusion that the nucleolus is less negative than the nucleus. The cell is thus a system with a more positive nucleolus, a negative nucleus, and a more positive cytoplasm. The outer solution which bathes the cell probably differs electrically from the cell protoplasm (as already pointed out from pH values, page 330). This means that differences in electric potential exist between nucleolus, nucleus, cytoplasm, and external medium. The living cell is thus the seat of electric forces. Keller terms it an accumulator, that is to say, an electric storage cell in which the nucleus is the cathode, or negative pole.

The staining of cytoplasm with acid dyes suggests that it is electropositive, as does also the idea that the nucleus is the cathode; yet both statements need indicate only that the cytoplasm is less negative than the nucleus. Other (cataphoretic) experimental findings show the cell to be negative as a whole. Evidence purporting to indicate that certain living cells may be positive has been advanced, but opinion and experimental

results are overwhelmingly in favor of all cells being negatively charged.

The fact that the cell is the seat of electric forces has led to speculation on electric interpretations of cellular behavior. The migration of chromosomes—which we have seen was regarded as a possible electromagnetic phenomenon (page 332)—has also been interpreted electrokinetically by Kuwada and Sugimoto. They suggest that the chromatin (chromosome material) of the resting nucleus and the poles (ends of the cell) are electronegative. The chromosomes are, therefore, at first repelled by the poles and gather at the center of the cell on the equatorial plate (Fig. 12d). Then, for some reason, the chromosomes become electropositive (one might just as well have the poles change their sign) and are now attracted by the poles.

Spek has obtained some interesting information on the electric forces which are possibly at play in the cell during mitosis. He finds that in the *Nereis* embryo (of several blastomeres), one end (pole) is alkaline and the other more acid. As each new blastomere is formed, it too has an acid and an alkaline end, so oriented that the acid side is toward the acid pole of the embryo as a whole. The egg and embryo thus show a definite acid polarity, which means a difference in potential.

Gross Manifestations of Potentials in Living Matter.—The severe electric shocks given by marine organisms, of which the ray fish (torpedo) and the electric eel are the best known, are examples of very high potential differences in living matter. The ray carries electrically charged living storage cells in his head which may deliver a potential of 30 volts. The electric eel may give a still higher voltage; that of *Gymnotus*, the South American eel, is said to reach a maximum of 1,000 volts, sufficiently strong to kill moderately large animals. The presence of a potential in the ray may be demonstrated by allowing the fish to rest upon a metal plate with another plate upon him. If the two plates are connected to a group of students, a bell, or an electric lamp, and the ray angered by twisting its tail, a substantial shock will be felt by the students, the bell will ring, or the light burn. On occasion, a 6-volt electric-light bulb may be burned out by the excessive voltage of the ray.

The most extraordinary feature of these high potentials in living systems is the fact that nature is here producing voltages

of a magnitude that cannot possibly be duplicated in the laboratory *without metals*, and we have no knowledge of the means by which nature accomplishes this.

The thought that potential differences exist between parts of organisms, *e.g.*, between leaf and stem, stem and root, has long prevailed. Some early experiments on plants were done by Buff (1854), Elfving (1882), and others, and more recently by Votchal and particularly Lund. The zoologists followed with determinations of potential differences in hydroids. The most extensive of this work is that of Lund. He finds that a continuous output of electrical energy is produced in living tissues. He believes that there is a definite relation among polarity, oxidation, and potential, and that the total electromotive force between two points is the algebraic sum of all the individual electromotive forces of the cells between which the potential is measured. The work of Lund has had to do primarily with fir trees, where he finds a potential difference varying from 30 to 200 mv. The growing point at the apex of the tree is positive to more basal parts. The external polarity, Lund says, is merely evidence of a complex but definite pattern of internal electric polarities, which serve to correlate the activities of one cell with another. There is also the possibility that "one of the functions of the continuous electric current which is directed upward in the wood is to supply electrical energy for electroendosmotic flow of sap" (see also page 379).

CHAPTER XIX

ELECTROKINETICS

Bacteria, fern spores, and nearly all living cells, when freely suspended in an aqueous medium, will migrate toward one pole or the other when they are subjected to the influence of an electric field. They must, therefore, possess an electric charge. If this charge is characteristic of cells, it may possibly play a part in their life and to some extent determine their activities. How far this last statement is true cannot as yet be definitely stated. It is, however, true that the electric charge on cells may be used to determine other very fundamental qualities which are directly responsible for the potentialities of cells and of organisms as a whole.

The electric charge on the surface of cells, and on nonliving particles as well, is a colloidal property which is grouped with others under the term *electrokinetics*.

Classification.—The colloidal state of matter has many physical properties peculiar to itself. Certain of these are electrical and manifest themselves in the movement of particles, or an aqueous medium, under the influence of an electric field. If the ends of two wires coming from a source of current are put into the colloidal dispersion of a metal or an oil or into a suspension of living cells such as bacteria, then the particles of metal, oil, or bacteria will move toward one pole or the other. The movement of colloidal particles in an electric field is known as *cataphoresis*, or *electrophoresis*. If, now, the particles are made to move through the liquid by mechanical means, *e.g.*, by allowing sufficiently large ones to fall through a column of water, an electric potential is produced. This phenomenon is known as the *Dorn effect*. It is, in a sense, the reverse of cataphoresis in that in one case mechanical motion is caused by an electric potential, while in the other an electric potential is produced by mechanical motion.

Suppose, now, that the particles are held still—we can imagine them clinging together and forming a porous disk—and an electric field is again applied; the water will then flow through the capillaries of the porous mass. This is known as *electroendosmosis*, shortened to *electrosmose*. If, again, matters are reversed, and the water is forced through the capillaries by mechanical means (hydrostatic pressure), a potential is produced. The electric force thus generated is known as a *stream potential*. It is the reverse of electroendosmosis.

Colloidal phenomena such as we have been considering are grouped by Freundlich under the term *electrokinetics*. To recapitulate: (1) Electroendosmosis (electrosmose) is the flowing of a liquid through capillary tubes under the influence of an applied electromotive force; (2) cataphoresis (electrophoresis) is the migration of solid particles in a liquid in response to an applied external electromotive force; (3) stream potential is the electromotive force set up by the mechanically impelled movement of a liquid through capillaries; and (4) the Dorn effect is the potential produced by the motion (falling) of solid particles through a liquid. Electroendosmosis and cataphoresis are forms of motion caused by an applied electromotive force, while stream potentials and the Dorn effect are forms of motion that produce an electromotive force.

Of these four phenomena, cataphoresis is the one that has been most studied, but electroendosmosis first held the attention of the physicists. Both were simultaneously discovered in one experiment more than a century ago, though it is doubtful if the experimenter understood the full significance of his findings. In 1807, the Russian physicist Reuss placed two glass tubes in a piece of wet clay, filled the tubes with water, and connected them to a voltaic cell. He observed that at the positive pole the water became milky because of the migration of the fine clay particles. At the negative pole, the water remained clear but increased in volume. The first observation was one of cataphoresis; and the second, of electroendosmosis. When the movement of colloidal particles in an electric field takes place in capillaries, a certain amount of electroendosmotic flow usually accompanies cataphoretic migration, because the same electric force that causes the colloidal particles to move will also cause the water, where it touches a wall or membrane, to flow.

Methods.—Electroendosmotic flow can be readily demonstrated by the simple apparatus shown in Fig. 155. A sack made of collodion is filled with and immersed in water. Two electrodes—the anode without and the cathode within the sack—connect the system to a source of potential. The sack is tightly corked, with an outlet for a glass tube. After current is applied, water very slowly rises in the tube because of electroendosmotic flow through the pores (capillaries) of the collodion membrane.

Cataphoretic flow may be observed by fastening two metal strips to a microscope slide, putting several drops of a colloidal suspension, *e.g.*, diluted milk, between the metal electrodes, and then placing a glass cover slip on top; but it is just this simple experiment which led so many earlier workers in the field, and still leads beginners, astray. In such a simply made chamber the cataphoretic flow is completely disturbed by the electroendosmotic flow. Results purporting to find cells positively charged are due to the use of such a primitive chamber.

A U tube, into the ends of which project two electrodes, suffices for direct macroscopic observation of cataphoresis (*A*, Fig. 156). The clouding up of the colloidal solution near one electrode in one end of the tube indicates the direction of flow. If a negatively charged arsenic trisulphide suspension is placed in a U tube, and a direct current of 110 volts applied through electrodes, within an hour the anode (positive) end of the tube will show a more dense color, indicating a higher concentration of the colloid, while the opposite negative pole has become clear, the water here having been robbed of its colloidal particles.

Similar in principle is the cataphoretic tube of Pauli (*B*, Fig. 156). It was designed primarily for the study of protein migration. Acid or alkaline protein is put in the lower part of the

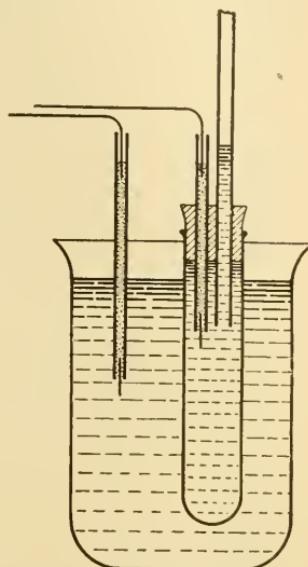


FIG. 155.—Demonstration of electroendosmotic flow of water; flow takes place from the outer positive pole, through the (collodion) membrane to the inner negative pole; rise of the water in the capillary tube indicates flow.

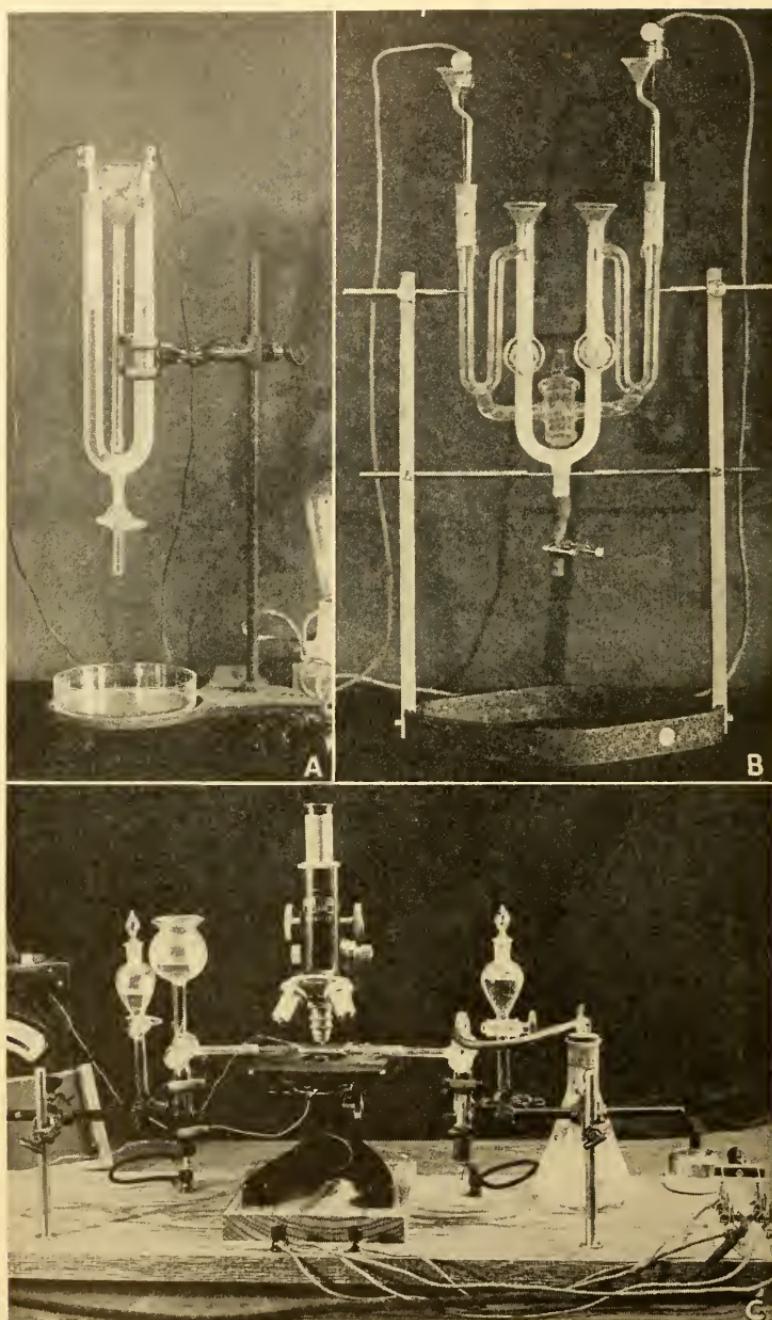


FIG. 156.—A. A U tube for demonstrating cataphoretic flow; the solution in the right arm of the U is opaque due to concentration of As_2S_3 at that (the positive) pole. B. The Pauli cataphoresis chamber for proteins. C. The Northrop-Kunitz cataphoresis chamber mounted on a microscope.

U tube. The central stopcocks are closed, and the remainder of the apparatus rinsed and filled with acid or alkali (of the same concentration). The stopcocks are then cautiously opened, and current applied to the two electrodes. Within an hour (using 70 to 75 volts), enough protein will have migrated toward one or the other pole to give a precipitate when sulphosalicylic acid is added. If the protein is acid, it will migrate to the negative pole; if alkaline, to the positive pole. Electrolyte-free albumin has a very weak negative charge and therefore migrates to the anode.

Most cataphoretic work involves the observation of individual microscopic particles. An original apparatus for such work

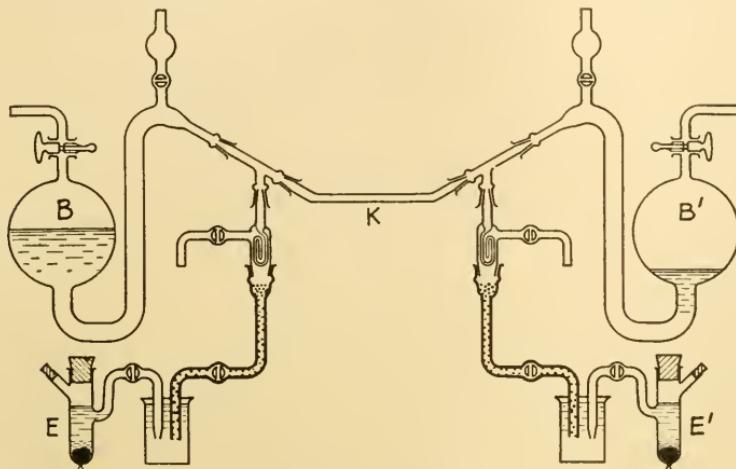


FIG. 157.—The Kruyt cataphoresis chamber.

is that shown in Fig. 157. In America, the Northrop-Kunitz model is extensively used (*C*, Fig. 156). In all cases, the apparatus consists of two electrodes and a central glass chamber. The latter rests under the microscope objective. Each end of the chamber is joined to glass tubes which lead to zinc electrodes through two-way stopcocks. When an external electromotive force is applied to the electrodes, the particles in suspension are seen to migrate in one or the other direction. The rate of migration is determined by timing a particle across a scale inserted in the microscope ocular. Comparative results may be expressed in terms of rate of movement in microns per second per volt per centimeter.

A number of factors are involved in the migration of a colloidal particle in an electric field. One of these is the electrokinetic

potential, *i.e.*, part of the total potential difference between the particle and its surrounding medium. If this value is desired, it may be calculated by a formula. It cannot be directly, electrometrically, measured. In addition to the potential on the surface of the particle, the field strength is a factor: it is a definite part (determined by the type of apparatus) of the total potential across the field. It is expressed in volts per centimeter and may be roughly measured by connecting a voltmeter to two platinum electrodes fused into the glass chamber. Voltmeters are shunted and are of high resistance so as not to receive the full flow of current. As the solution in the cataphoresis chamber is of equally high resistance, the current will be divided; the part going through the voltmeter will reduce the recorded potential.

A more exact calibration of the cataphoretic chamber involves measuring the cross-section area of the cell; then, by measuring the current during experiments and the specific resistances of the solutions used, the field strength in the cell may be calculated by Ohm's law for each experiment (method of Abramson and Moyer).

The experimental difficulties attendant on precise work in cataphoresis are greater than here indicated. One problem to be solved is the correct level in the chamber at which the rate of migration of the particles should be measured. The rate is different at different levels, because the aqueous medium carries on an independent electroendosmotic flow, while the particles migrate cataphoretically. More than this, the electroendosmotic flow of the water is in one direction (to the cathode) close to the walls of the chamber, while in the center of the chamber there is a hydrodynamic flow of the water in the opposite direction due to a return current. This is true because the chamber is a closed one, and the water must follow a circuit. One of these currents opposes and the other aids the movement of the particles. Figure 158, in which the thickness of the chamber is greatly magnified in proportion to its length, illustrates the situation. Either the average of a number of readings at different levels from top to bottom must be taken, or all readings must be made at one of the two stationary levels which lie midway between the opposing currents. (Even in this latter case, it is well to average the rates of travel of a particle in both directions, by reversing the polarity of the electric circuit.) The two stationary levels

are at 0.21 and 0.79 of the total depth, from the top or bottom of the chamber.

The effect of the electroendosmotic flow of the water on the migration of the particles is nicely illustrated in the case of mushroom spores, the specific gravity of which is just great enough to cause them to fall slowly; in so doing, they pass through the successive layers of flow (electroendosmotic and hydrodynamic) of the water. When the spores, in falling, reach the upper layer of no flow of water, they, being negative, move to the anode. This represents motion due to their own electric charge, which is weakly negative (in a salt solution of pH 8.2). As they fall

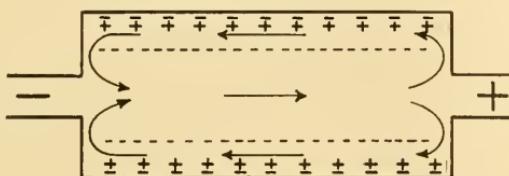


FIG. 158.—An enlarged longitudinal section (with depth exaggerated) of a cataphoresis chamber showing endosmotic flow of water, and the two quiet levels (dotted lines).

farther, they first pass through water which flows with them (in the center of the chamber) and increases their speed. They then reach the lower, quiet layer of water and finally the water at the bottom of the chamber, which opposes them by its greater flow in the opposite direction to such an extent as to reverse the direction of their migration.

Theory.—If water fills a glass capillary tube or a porous clay disk (which is essentially a mass of capillary tubes), and if two wires coming from a source of current are placed one in each end of the tube or one on each side of the porous disk, the water will move through the tube or through the disk toward the cathode or negative pole. The German physicist Quincke was the first to suggest that this extraordinary electroendosmotic migration of a liquid through capillaries under the influence of an applied potential is due to an electric charge which lines the walls of the capillary in such a way that electricity of one sign is in immediate contact with the solid wall, while electricity of the opposite sign, equal in quantity to the first, forms a second layer lying near the first. The German physicist Helmholtz offered

a mathematical expression for Quincke's suggestion. The electric surface is known as the *Helmholtz double layer*.

Numerous mathematical and theoretical hypotheses have been advanced in explanation of the electric layer on the surface of colloidal matter, but these are not needed for a simple interpretation of it. Let us first consider a glass capillary tube in which there is water flowing under the influence of an applied electromotive force. Quincke and Helmholtz assumed that the inner wall of the tube is lined with negative charges which adhere to the wall (Fig. 159). Adjoining them are positive charges, equal in number and lying within the water. The negative charges

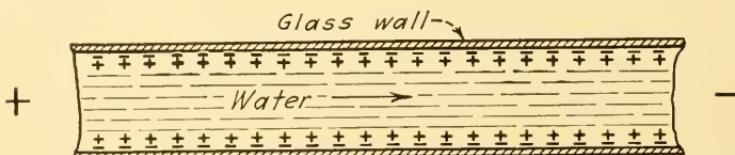


FIG. 159.—The Helmholtz double layer in a glass capillary tube filled with water.

adhere to the glass wall and cannot move; the positive charges are free to move, and, being positive, they travel toward the negative pole of the applied electromotive force, carrying the water, of which they are a part, with them. The moving positive charges in the water glide by the stationary negative ones on the glass.

The positive charges are H^+ ions, and the negative ones are OH^- ions. (It appears that the H^+ and OH^- ions are subsidiary here to the H^+ and HCO_3^- ions in ordinary water.) Helmholtz could not have anticipated this, as ions were unknown in his time. Water, being a weak electrolyte, is slightly ionized. The glass becomes negatively charged apparently because of preferential adsorption of negative (OH^-) ions. The moving water is positive, because each OH^- ion adsorbed by the glass leaves one H^+ ion—its mate—free in the water. The positive H^+ ions, being free to move, are attracted by the negative pole of the electric field. The speed at which the water travels through the tube under the influence of an external electromotive force depends on the potential of the current applied and on the potential of the Helmholtz layer. This latter is, in part, determined by the distance δ between the two layers of negative and positive ions. The dielectric constant, or the electric ionizing

capacity of the liquid, is also a factor. Opposing these are mechanical forces, such as friction, which depends on the viscosity of the liquid and the size of the capillary tube. The relationship of these factors was mathematically expressed by Helmholtz. The original formula has been modified several times. Pellar added the dielectric constant of water. The formula as now given is

$$V = \frac{\xi E D r^2}{4\eta l} ,$$

where V is the volume of liquid transported per second through a tube of radius r and length l ; ξ , the difference in potential between the stationary charged layer and the outer Helmholtz layer (in electrostatic units); E , the external electromotive force applied to the electrodes at the ends of the capillary tube; D , the dielectric constant of the liquid; and η , the coefficient of viscosity of the liquid.

So far, we have centered our attention on the electroendosmotic flow of water and the nature of the charge on the walls of the capillary. We may transfer the same ideas to the migration of individual colloidal particles through water under the influence of an applied electromotive force.

We may imagine the glass capillary in Fig. 159 broken up into minute bits which are suspended in the water. The same double layer of ions will then exist at the surface of the glass as before, with negative ions tightly adhering to the glass and positive ones close by but free in the water. The glass particle, like the glass capillary tube, is negatively charged but being now itself free to move travels to the positive pole.

A Helmholtz double layer or a modified form of it is usually present at the surface of all colloidal particles (Fig. 76). The particles of any one colloidal suspension are all of the same sign. Whether the particles are negative, as are those of glass, gold, and living cells, or positive, as are those of copper, lead, and iron hydroxide, depends on whether anion or cation is preferentially adsorbed to the surface of the particle. (Adsorption of ions is generally assumed to be the cause of the charge (see page 109), but in the case of proteins dissociation of ions from the particle may be responsible.)

Why some colloidal particles preferentially adsorb negative, and some positive, ions is not definitely known. Hypotheses have been advanced; for example, the negative charge is presumed to be due to the high dielectric constant of water; but there are many kinds of particles that are positive in water. Preferential adsorption may rest upon a weak initial charge of the metal, of opposite sign to that of the particle; thus, a positive metal would adsorb negative ions, and a negative particle would result. More convincing is the hypothesis of relative solubility. If a metal is soluble in water, an environment of its own ions will be formed around it.

Not only are colloidal suspensions positive or negative, but charges produced by the Dorn effect may be positive or negative. Burton has found that lead shot dropped through water, alcohol, benzene, toluene, and xylene carry down a negative charge; through turpentine and carbon tetrachloride, a positive charge; and through ether, no charge.

The Helmholtz formula, as given above for electroendosmotic flow, also applies in a modified form to the migration of particles. The formula for cataphoretic migration is

$$U = \frac{\xi HD}{4\pi\eta}$$

where U is the velocity of the particle in centimeters per second; and H , the field strength in electrostatic units per centimeter, i.e., the potential gradient, or drop, per centimeter. In the development of this form of the original formula, a number of workers, notably Perrin, have played a part. A comparison of the two formulas reveals that E/l in the one first given for electroendosmotic flow has been replaced by H . H is the potential gradient, or drop in voltage, per centimeter and is therefore identical with E/l , for E is the total external electromotive force, and l , the distance between the poles.

The character of the immediate environment of colloidal particles has been the subject of much speculation. Helmholtz thought it to be a compact double layer of charges, but he was the first to point out that an equal number of opposite charges would leave the particle electrically neutral, a condition that would not bring about movement in an electric field. He mentions this and

then points out how it is that an electrically neutral particle can move. He says:

On the whole, the algebraic sum of the two equals zero, and the center of gravity of the complete system, solid particle, and surrounding positively charged fluid layer taken together cannot be moved by the electric forces which arise from the potential fall in the liquid through which the current passes. However, the electric force will tend to bring about a displacement, relative to each other, of the positively charged fluid layer and the negatively charged particle, whereby the fluid layer follows the flow of positive electricity while the particle moves in the opposite direction.

Thus the particle, with its adhering layer of negative ions (if it is a negatively charged particle), slips from under the outer layer

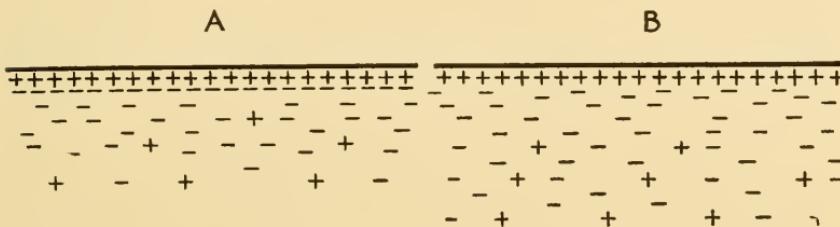


FIG. 160.—Schematic representations of the diffuse layer of ions surrounding colloidal particles.

of freely suspended positive charges (Fig. 77). In spite of this rather satisfactory explanation, other difficulties arose which led first Billitzer, then Gouy, to advance the hypothesis that the inner layer is closely adherent to the surface of the particle, as Helmholtz stated, while the outer layer is not a single layer at all but a cushion or cloud of ions. We thus, in another way, escape the difficulty that arises when the colloidal particle is viewed as a neutral condenser made up of two compact layers of charges, equal in magnitude and opposite in sign. The concept of a diffuse ionic cloud surrounding colloidal particles is now generally accepted. The exact arrangement of the ions in the diffuse layer is obviously not known. There may be a cloud with the Helmholtz double layer more or less clearly marked (*A*, Fig. 160), or there may be a closely adhering single layer of one electric charge (which determines the sign of the particle) and an adjoining diffuse layer of the opposite charge (*B*, Fig. 160).

The charges that constitute the Helmholtz double layer or the Gouy cloud are assumed to be ions. In the case of the electro-

endosmotic flow of water through capillaries, the ions are apparently the H⁺ and OH⁻ ions of the water. On colloidal metal particles, the ions could conceivably also be those of water, but the evidence is strongly against this possibility; it indicates that the ions are those of a salt of the metal. Pauli has described the situation for solid colloidal suspensions. The ionic environment of colloidal gold particles consists of a compact inner layer of ions of gold chloride, AuCl₂⁻ (or AuCl₄⁻), and a diffuse outer layer of H⁺ ions.

The thickness of the ionic atmosphere around colloidal particles (*i.e.*, that part of it which determines the charge on the particle) varies from the unimolecular dimension of the compact Helmholtz double layer (from 1 to 20 A. U., or about 1 $\mu\mu$) to the hundred or more molecules in the depth of an ionic cloud. The depth of the latter has been calculated to be 0.96 m μ for a N/10 solution of salt, 9.6 m μ for a N/100 solution of salt, and 1,010 m μ for pure (conductivity) water.

The potential at the surface of a colloidal particle—the so-called electrokinetic potential—represents the difference between the electric pressure on the colloidal particle and that in the surrounding medium. This potential difference is of the order of 40 to 50 mv. (0.050 volt). The potential at the surface of colloidal gold has been found to be 45 mv. (under the conditions of the experiment). The value depends upon the thickness of the ionic cloud, which, in turn, is dependent upon the concentration of the surrounding electrolyte. The electrokinetic potential ξ can be calculated in terms of the rate of movement U .

$$\xi = \frac{4\pi\eta U}{HD}$$

As the Helmholtz potential ξ is the difference in potential between the adhering layer of ions and that of the immediately surrounding ionic atmosphere, it is evident that this potential difference will depend upon the amount of the surrounding medium which is to be considered a part of the immediate environment of the particle. In other words, if we are measuring the drop in potential between two electrodes immersed in a common liquid, we must determine just where one electrode with its potential ends, and the other with its potential begins.

Freundlich and Rona suggested that only a part of the outer diffuse layer of ions is actually mobile; that is to say, the colloidal particle carries with it not only the innermost single and closely adhering (Helmholtz) layer but also some of the outer ions. This being true, the particle, regarded as an electrode, becomes somewhat larger than its actual boundary—its surface of metal. The adhering ions are probably attached to the particle by secondary forces (secondary valence, adsorption, van der Waal forces). Electric charges (in this case, ions) are subject to heat motion. Near the surface of the particle the electrostatic forces

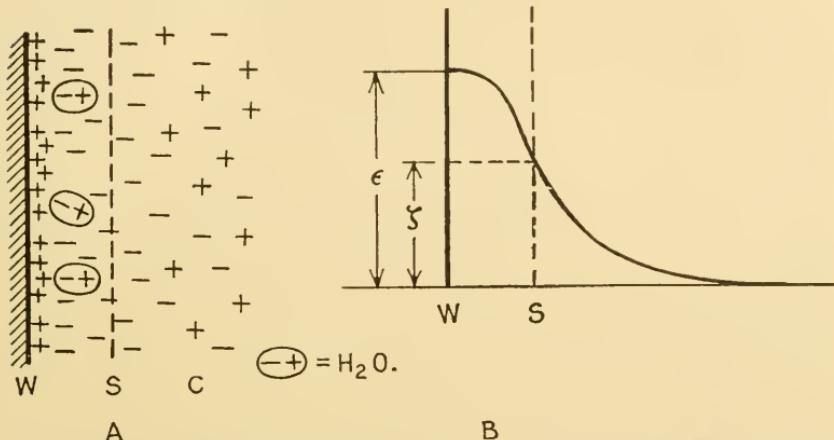


FIG. 161.—Potential distribution at the surface of a particle. W is the wall of the particle; S the plane of slippage; C the outer free cloud of ions; ζ the electrokinetic potential; and ϵ the thermodynamic potential. (After H. Müller.)

are so large that the motion due to temperature is negligible. This area extends out into the fluid for a short space until at a sufficient distance the ions overcome the electrostatic forces and move freely. The interface, where the inner adhering ions move or slip with the particle against the outer free ions, is known as the *plane of slippage* (S , Fig. 161A).

If our hypothetical particle were at absolute zero, it would be found to possess a rigid Helmholtz double layer of positive and negative ions equal in number and placed within ionic diameters of each other. But owing to the heat motion of the ions, at ordinary temperatures, the ions outside the plane of slippage distribute themselves in a *diffuse* double layer (corresponding to the density of the atmosphere about the earth). Far off in the body of the solution (C , Fig. 161A) the number of positive and

negative charges balance each other, but in the diffuse double layer there is, over a time average, an excess of ions of one sign in any unit volume. The difference of potential (ζ) across the double layer depends upon the thickness of this ionic atmosphere (which in turn depends upon the concentration) and the number of adsorbed charges within the plane of slippage per unit area. It is ζ which determines the speed of a particle moving under the influence of an electric field. It is, therefore, ζ that should express the potential in the Helmholtz formula.

In addition to the ζ potential there is the ϵ or thermodynamic potential. ϵ is usually considered to be the *total* potential between the surface and the body of the solution, whereas the electrokinetic potential ζ is the potential drop between the plane of slippage and the body of the solution. The curve in Fig. 161B shows the fall of these potentials with distance from the particle. ζ and ϵ do not bear a clear relationship to each other; ϵ is treated by thermodynamics, whereas ζ is not. The Gouy theory deals only with ζ . ζ is much more sensitive to the effects of salts than ϵ .

The field strength near the particle is, on the basis of estimates by H. Müller, exceedingly great. For this reason the "layer" of ions is greater than ionic dimensions; and for this reason all heat motion of the nearer ions is nullified. Furthermore, there are probably distortions of the ions, and also orientation of the water molecules. These latter, functioning as electric dipoles (Fig. 177), will be held in definite orientation (Fig. 161A).

Abramson has been able to show that when quartz particles are placed in certain protein solutions they behave like protein molecules; *i.e.*, they become coated with a film of protein. Since these quartz particles are of microscopic dimensions, whereas protein molecules are not, use of this method facilitates the investigation of proteins by the microscopic method of cataphoresis. By measuring the cataphoretic mobilities of these protein-coated particles, Abramson has calculated their charge by the Gouy theory and also thermodynamically. A comparison of the two values obtained by different methods reveals essential agreement, thus confirming the Gouy theory.

In laboratory practice, it is more convenient, and for most purposes as satisfactory, to express cataphoretic observations in terms of rate of migration (in microns per second per volt per

centimeter) rather than in terms of the electrokinetic potential. But if the potential of the particle is desired, one can, by making permissible assumptions in regard to the dielectric constant, the viscosity of the aqueous medium, and the potential gradient, derive the potential directly from the rate of migration simply by multiplying the latter by a factor. Obviously, this factor will have a different value under different conditions. For average laboratory conditions, it has been established as 12.6. If the rate of migration of a droplet of butterfat (in milk) is 3.6μ per second per volt per centimeter (the total potential of the line being 110 volts; the potential gradient in the chamber, 6 volts per centimeter; and the actual observed rate, 21.6μ per second), then, by applying the factor 12.6, we obtain

$$12.6 \times 3.6 = 45 \text{ mv.,}$$

which is the potential on the surface of the particle. With standardized equipment and constant temperature, the factor, once established, will hold for subsequent work.

The foregoing account of the electric properties of colloidal particles ascribes their behavior to their environment, which adds further evidence to the fact that the properties of colloidal systems are determined primarily by the third phase, or interface. Without wholly departing from this viewpoint, there are those who prefer regarding a colloidal particle as a colossal polyvalent ion. Thus, McBain says that the behavior of micelles is exactly like that of the ions of an ordinary electrolyte. This viewpoint is also held by Pauli in regard to both protein molecules (or micelles) and metal particles. Mukherjee states that the migrations of ions and of colloidal particles are fundamentally similar, but an ion and a colloidal particle are not identical as chemical entities. There is a similarity between colloidal particles and ions, but the differences are equally pronounced, particularly in that property which best characterizes both, *viz.*, charge. The charge on colloidal particles does not seem to be related to that on the corresponding ions. Metallic ions in general are positively charged, but colloidal dispersions (Bredig dispersals) of them may be positive (as are copper and iron) or negative (as are platinum, gold, and silver). While the sign of the charge of colloidal particles appears to have no relation to that of the ion, the mobility of the relatively large particles is of the same

order of magnitude as that of ordinary electrolytic ions; but this is to be explained on the basis of high valency.

In the case of proteins, the analogy between colloidal particles and ion is perfect in regard to the sign of the charge, as we shall see in a moment.

Charge and Potential.—It is necessary to distinguish clearly between charge and potential; the former is often carelessly used to indicate the latter. Potential is expressed in volts; charge, in coulombs, or electrostatic units; potential is pressure; charge is quantity. Bikerman showed that the coagulation of a number of different solutions takes place at the same potential. Were charge responsible, diverse values would result because of the different dielectric constants of the solutions. There is a definite relationship and interdependence between charge and potential, as shown in the following formula for a plane condenser (such as are colloidal particles):

$$\zeta = \frac{4\pi\sigma\delta}{D}$$

where ζ is the potential; σ , the surface charge per unit area; δ , the distance between the two layers of charges; and D , the dielectric constant.

The distinction between charge and potential finds application in biology in some calculations by Abramson from data of Northrop and deKruif and Mudd and Joffe for the ζ potential of bacteria. The results show that the *charge* on the bacteria does not decrease as potential drops and may even increase (with increase in salt concentration); rate of agglutination increases with fall in *potential*.

Reversal in Charge.—It was difficult to explain why one metal is positively and another negatively charged; selective adsorption and relative solubility were the suggestions. Now comes the task of explaining why a metallic colloidal particle reverses its charge in a changed environment. Again use is made of adsorption. A negative particle adsorbs positive ions and becomes itself positive. Most substances when colloidally dispersed can be of one sign or the other, depending upon their environment (salt concentration, acidity, etc.). Examples of this are numerous. Burton has reversed the sign of the charge on a number of metals. The following table indicates the effect

of strongly adsorbed aluminum cations on the surface of negative silver particles. The direction (therefore the sign of charge), the rate of migration (therefore the potential), and the stability of colloidal silver particles are determined by the concentration of the aluminum salt.

Mg. Al/l.	Cataphoretic velocity, $\mu/\text{volt/cm./sec.}$	Stability of the silver dispersion
0.	3.30 to anode	Permanently stable
0.19	1.71 to anode	Flocculates in 4 hr.
0.365*	0.	Flocculates immediately
0.38	0.17 to cathode	Flocculates in 4 hr.
0.63	1.35 to cathode	Not completely flocculated after 4 days

* Calculated.

The adsorption of ions of high valence is the probable cause of reversal of sign in the case of metal particles. This is illustrated by the fact that negative colloidal gold may be made positive by Al^{+++} , Fe^{+++} , La^{+++} , and Th^{++++} but not (readily) by bivalent metals.

Reversal of the charge on proteins is due to other properties than ionic adsorption. Proteins have the capacity, when associated with acids or alkalies, to assume a negative charge when on the alkaline side of neutrality and a positive one when on the acid side. This *amphoteric* property of proteins, as illustrated by the sign of the electric charge, is due to their capacity to form salts with both acids and bases. In the presence of acids, proteins form salts in which the protein ion is positive; and in the presence of bases, they form salts in which the protein ion is negative. Thus, if A stands for albumin, then $A + \text{HCl} = A^+\cdot\text{Cl}^-$ (albumin chloride), and $A + \text{NaOH} = \text{Na}^+\cdot A^-$ (sodium albuminate). Albumin, therefore, becomes a positively charged ion in an acid solution and a negatively charged ion in basic solution. If, now, a protein particle is in an acid solution, it should, as an ion with a positive charge ($A^+\cdot\text{Cl}^-$), travel to the negative pole of an electric field; this it does. In an alkaline solution, it becomes negatively charged ($\text{Na}^+\cdot A^-$) and travels to the positive pole. It is, therefore, necessary only to change the acidic or basic condition of a protein solution in order to

change the sign of the charge of the protein ions, provided that the point of change (specific pH value) is passed.

Fat globules in milk are coated with a stabilization membrane which is probably protein (caseinogen). If this is true, then acid milk should be positively charged, and basic milk negatively charged. The fat droplets of pure milk are usually negative. The pH value of fresh milk is about 6.6. If acid is added until the pH value falls below 4.5, the globules become positively charged and migrate to the cathode. The direction of migration reverses at a definite pH value (4.55) and the rate of migration becomes progressively slower as this value is approached. In the following table is recorded the change in rate of travel of the fat (protein coated) particles in milk with change in pH.

CATAPHORESIS OF MILK

pH	Sign of charge	Observed velocity, sec. to cover 0.5 mm.	Calculated velocity, mm./sec.
3.72	+	12.8	3.9×10^{-2}
4.05	+	20.8	2.4×10^{-2}
4.27	+	48.8	1.0×10^{-2}
4.45	+	116.6	0.4×10^{-2}
4.63	-	69.4	0.7×10^{-2}
4.80	-	36.2	1.4×10^{-2}
5.23	-	33.4	1.5×10^{-2}
5.91	-	23.6	2.1×10^{-2}
6.24	-	22.9	2.2×10^{-2}
6.47	-	21.6	2.3×10^{-2}
6.64	-	20.1	2.4×10^{-2}
6.98	-	19.6	2.5×10^{-2}
7.17	-	17.3	2.8×10^{-2}
7.38	-	16.3	3.0×10^{-2}
7.73	-	15.6	3.2×10^{-2}
8.04	-	11.7	4.2×10^{-2}

The foregoing table may be expressed as a curve (Fig. 162).

The point at which particles in colloidal suspension reverse the sign of their charge is a very characteristic one and is specific for many substances. It is known as the *isoelectric point*. Proteins can sometimes be identified by it. It is usually expressed in terms of acidity (pH). The isoelectric point of milk is pH 4.55. This may be taken as convincing evidence that the

coating on milk globules is protein, for the isoelectric point of casein is 4.6.

Isoelectric Point.—The isoelectric point was defined by William Hardy as the point of no migration in an electric field; it became also that point on the acid-alkaline scale at which a solution of albumin flocculates. Hardy found that an acid

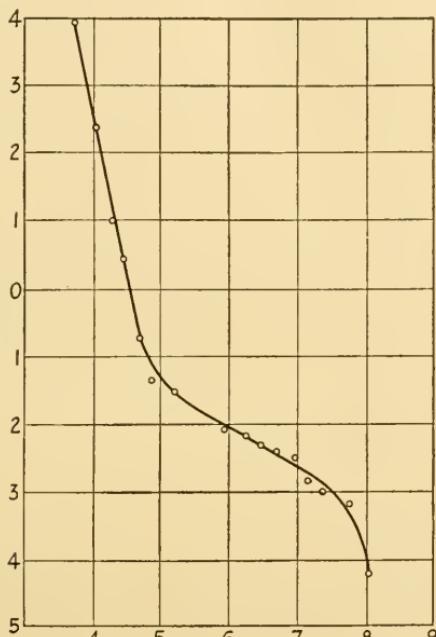


FIG. 162.—Change in rate and direction of migration of milk globules with change in pH: ordinates are calculated comparative rates of migration; abscissae are pH values: the pH of fresh milk is 6.6, its isoelectric point is 4.55.

solution of albumin migrates to the negative pole and is, therefore, positively charged, while an alkaline solution migrates to the positive pole and is therefore negatively charged. He also found that on both the acid and the alkaline side, a solution of albumin is stable; but that at an intermediate point, the suspension settles, or flocculates. Hardy stated that a colloidal solution is stable only when the dispersed particles possess a charge, and the point of zero potential, or "point of least density of electric charge," he called the isoelectric point. The particles are decharged because of some change in their environment. As this change is often one of acidity, that is to say, acidity is a measure of it, it is convenient to express the isoelectric point in

terms of acidity. The pH value at which latex (rubber) particles do not migrate is, as determined by Moyer, about 4.1 for certain species of plants. The isoelectric point is, therefore, expressed as pH 4.1.

Other properties of colloidal suspensions often change at the isoelectric point. Stability is one of these. At the isoelectric point, most colloidal suspensions settle out. The point of minimum or maximum turbidity of a colloidal dispersion (minimum where the suspension was originally coarse and has cleared by settling or rising of the particles; maximum, where the suspension was originally fine and has become cloudy because of aggregation of the particles) may indicate the point of zero potential and therefore the isoelectric point. Only in so far as the minimum or maximum value of a selected property of a solution indicates the point of zero potential, and therefore the point of no migration in an electric field, is it the isoelectric point.

The isoelectric point is rarely a point but a zone; that is to say, if the "point" at which particles will not migrate in an electric field is determined and expressed in pH, it will be found to be a range, say between pH 4.4 and 4.6, in which no migration occurs. There is the further fact, discovered by Powis, that coagulation does not take place only at zero potential but may occur at a higher value. The potential at the surface of colloidal particles is usually, in a stable solution, about 40 to 50 mv. Precipitation and allied phenomena may occur at any potential below 30 mv. At this value, an oil emulsion, which had an original potential of 46 mv. at the surface of its oil globules, may separate. Rather than refer to so high a potential value as the isoelectric point, especially as the "point" is a long range, it is instead termed the *critical potential*. Northrop found that certain bacteria agglutinate at 11 mv.; this is their critical potential. Usually, the potential at which a system becomes unstable, *i.e.*, the critical potential, is of more significance than the isoelectric point. It better characterizes the system.

While isoelectric points and reversibility are possessed by all types of colloidal systems, they are primarily characteristic of amphoteric substances such as protein and lecithin, which can be reversed with the hydrogen ion. It was in connection with the cataphoretic behavior of albumin that the term isoelectric point was coined. The property is not so typical of colloidal

metals. They cannot usually be reversed by the hydrogen ion, requiring special ions of nearly always high valency.

Stability.—The precipitation of colloidal metals, the flocculation of albumin, the coalescence of suspended droplets (the “breaking” of an emulsion), the coagulation of blood, and the agglutination (clumping together) of bacteria—in brief, nearly all colloidal precipitation phenomena—are, at least in the major aspects of their behavior, due to a reduction in the surface potential of the particles. (It should be emphasized again that the potential and not the charge is responsible.)

Usually, it is potential which is primarily involved in determining stability. Burton says that mutual electric repulsion (*i.e.*, ζ potential) is wholly responsible, certainly in the case of metals. Freundlich, Pauli, and others, without actually dissenting from this viewpoint in its fundamental aspects, attribute stability to a protective envelope of ions. The distinction is slight—a potential is recognized in both cases; but in the one (a protective ionic covering), the particles may strike in their random movement but do not adhere; while from the other viewpoint, they are kept from striking by their mutual electric repulsion.

Kruyt and deJong lay emphasis on hydration (page 147) which very likely plays a prominent role in the stability of proteins, though Kruyt says that it is not sufficient.

Whatever the nature of the environment of colloidal particles may be, when it is removed, the particles come into contact, adhere, and settle out.

IN THE LIVING WORLD

Electroendosmosis.—The passage of water through living membranes is an important event in the life of an organism. It is often neglected in studies on permeability where attention is usually centered on the passage of salts. The passage of water through a membrane under the influence of an electric potential is electroendosmosis. It may be the force responsible for the transference of water through the living membrane. S. Mudd has shown that when a mammalian membrane (mesentery and pericardium of dogs, rabbits, and human beings), bathed in a dilute buffer solution, is traversed by an electric current, liquid is caused to stream through the membrane toward the cathode

when the pH value is on the alkaline side and toward the anode when on the acid side of the reversal (isoelectric) point.

The one-way diffusion of water through the membranes of tissues, as in the case of the inner wall of the intestines, is a well-known but not fully understood phenomenon. Electro-endosmosis is a possible interpretation of it. The membrane is a mass of capillaries, each presumably lined by a Helmholtz double layer (Fig. 163). If there is a potential gradient across the membrane, water will flow in one direction only.

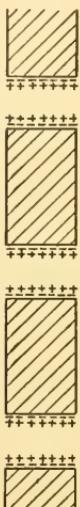


FIG. 163.—
Electric charges lining
the pores of a
membrane.

Electroendosmosis has also been resorted to as an explanation of gland secretion. Glands exhibit anomalous flow in that substances in solution move from a region of low concentration in the gland to one of high concentration in the surrounding tissue. For example, the nectary of a flower continues to secrete nectar even when its own tissue actually contains less nectar than the solution outside the gland in the nectary. Possibly, electroendosmosis is the force responsible, for then, under the influence of an electric potential, a solution will flow contrary to simple diffusion laws.

N. Marinesco has applied electrokinetic forces to the problem of the ascent of sap in plants, a problem that has long interested botanists and never been conclusively settled. He has submerged a bundle of xylem vessels (the sap-conducting capillaries of woody plants) in an electrolytic solution and applied an external electromotive force to the extremities of the tubes, thus provoking a displacement of the liquid which traverses the tubes. If a difference in potential exists between the stem and root of a plant, it is possible that the rise of sap may be an electroendosmotic phenomenon. That such a potential difference exists Marinesco has shown to be true, as have others. He finds a potential difference of 400 mv. between the stem and root of Fuchsia. This electromotive force may be produced by the upward flow of the sap (*i.e.*, it is a stream potential). Obviously, if this is true, then the electromotive force cannot be the cause of the rise of the sap. Which is cause and which effect it is impossible to say. However, as the two phenomena are quite

evidently associated, it should be possible to accelerate the ascent of sap by applying an electromotive force to the terminals of a plant (Fig. 164). Whether we thus aid or retard the movement of sap will depend on the sign of the charge of the flowing water. In the case of glass capillaries, we found it to be positive (page 364); in other capillaries it may be the same or it may differ. Most work on cellulose indicates that the capillary

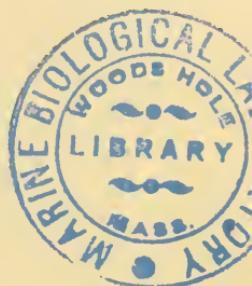
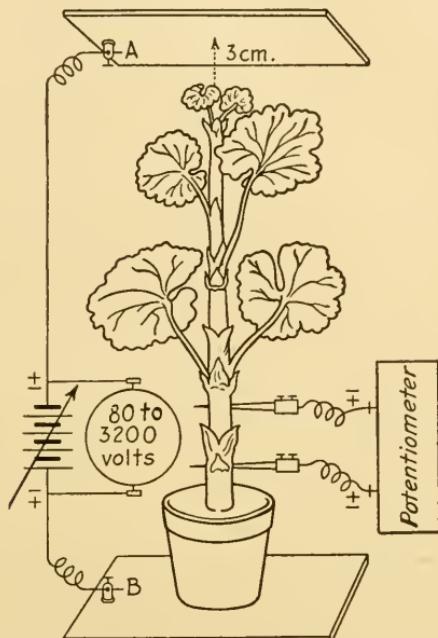


FIG. 164.—A plant in an electrical atmosphere which brings about an increase in flow of sap. (*From N. Marinesco.*)

water is positive. In the experiment of Marinesco no direct connection between the applied field and the plant is necessary. The latter is simply put in an atmosphere of electricity.

If it is true that the rate of flow of sap is accelerated by an applied electromotive force, then an applied external electric field should be expected to have an influence on the rate of growth of a plant. Such experiments in *electroculture* have been done (page 333), with, however, varying results.

Cataphoresis.—Relatively little work has been done on the cataphoretic migration of whole plant cells, except for bacteria. There are some observations by Winslow on yeast and the green unicellular alga Chlorella and by others on fungal spores and

pollen grains. The charge in every case is negative. Here and there in the literature, one reads that certain cells are positively charged or that certain cells appear to be positive at times and negative at other times. Most results that indicate a positive sign of the charge on living cells are erroneous in so far as they are due to one of two very common faults in cataphoretic research work, *viz.*, failure to observe the level at which movement takes place and failure to measure pH. The strong electroendosmotic flow of the water in a cataphoretic chamber may cause a weakly charged negative particle to travel to the negative pole against its own electrokinetic attraction for the positive pole. Acidity is another source of error. Acid added to a suspension of electro-negative bacteria may reverse the charge at reactions on the alkaline side of their isoelectric points. That colloidal particles may change sign when salt or acid is added has been seen. The change usually occurs at an abnormally low (or high) isoelectric point, *i.e.*, at a salt concentration or pH value unlikely to occur in nature. Should a cell have an isoelectric point near neutrality, then the possibility of its being either negative or positive in the natural state would have to be granted, but as yet there is little evidence that living cells are positively charged in their normal environment. Comandon has observed red blood cells migrating to the anode (the cells therefore are negative) and trypanosomes migrating to the cathode (and therefore positively charged).

Spermatozoa and Spirochaeta are cells that have most often been reported to be positively charged. Why they should be so it is difficult to understand. Koltzov and Schröder found that some of the spermatozoa of rabbits are negative, some positive, and some show no migration in an electric field. They believe that difference in sign of charge may be associated with chromosome and sexual characters. In order to put all such data to the test of modern and precise technique, S. Mudd studied the sperm from man, bull, ram, rat, rabbit, and guinea pig and found it in every case to be negative. He also applied the same careful methods to spirochaetes (bacteria) which had been described as positively charged. These, too, he found to be negative.

Katsuma Dan, working on the eggs of the sea urchin and using a special technique because of the large size of the eggs when compared to colloidal particles, found the charge of unferti-

lized, fertilized, and cleaving *Arbacia* eggs to be negative under all conditions, the potential being of the order of 30 mv.

If it is actually true that certain cells are positively charged under normal conditions, then one of two conclusions is unavoidable: either the cell is not covered with a protein; or, if a protein, it is a very unusual one, in that its isoelectric point, in terms of pH, is above that of most proteins and on the alkaline side of the usual pH value of physiological solutions. Most proteins form salts in which the protein ion is the anion (negative) above a pH of 4.0 or 4.5. The pH value of physiological solutions is 5.5 to 7.4. A protein with an isoelectric point in this range is rare; and that is where the isoelectric point of proteins that cover positively charged cells would have to be. The coating on cells need not, of course, be protein, though there are many reasons to believe that it usually is (see page 375).

While it is not likely that plants differ from animals or that groups or strains of organisms differ from each other in the sign of the charge of their cells, it does appear that genera, species, and pathological strains of plants and animals differ very characteristically in the magnitude of the potential on their cells and particularly in their isoelectric points; in other words, the potential on living cells appears to bear a definite relationship to certain biologic (generic and pathogenic) properties.

Abramson found that the rate of migration of red blood corpuscles in an electric field was greatest in the dog and least in the rabbit. He obtained the following order: dog > rat > mouse, cat > monkey > man > guinea pig > opossum > pig > sloth > rabbit. The corpuscles of the dog migrate the fastest and therefore have the highest electric potential.

Bacteria probably require an electric charge in order to remain in suspension and therefore to keep alive. It appears from work by S. Mudd that their potential also aids them in their penetration of mammalian membranes and therefore in their passage from cell to cell. It might then be asked, Does electric potential determine pathogenicity; is the virulence of bacteria in any way associated with their surface potential? A definite answer to this question cannot be given. Virulence, as we know it, is a very complex property, but the pathogenicity and the electric potential of bacteria have apparently been correlated in at least one case.

Findings by Falk indicate a possible relationship between the virulence of bacteria and their electric charge.

There are four known types of pneumococci, each of which causes a distinct kind of lobar pneumonia. The types are numbered 1 to 4. From cultures of known pneumococcus types, Falk took samples and measured their respective cataphoretic rates. He found that type 3 moved the fastest when in a cataphoretic chamber under the influence of an electric field; type 1 was next; then type 2; and type 4 was the slowest. White mice were given intraperitoneal injections of one of the four types of bacteria, and the time of their death after injection noted. It was found that the sequence of decreasing virulence for mice of the four types of pneumococci was the same as that of the potential of the bacteria, *viz.*, 3, 1, 2, 4; in other words, the higher their electric charge the more deadly the bacteria. Whether this result was a matter of chance or there actually is such a striking relationship between the virulence and the interfacial potential of bacteria cannot be said. Further evidence obtained by Falk shows that the mortality rate among 720 cases of human beings suffering from lobar pneumonia decreases from 44.3 to 13.2 per cent with the type of pneumonia in the order 3, 1, 2, 4, which is that of decreasing potential. There are many kinds of bacteria, some of which are deadly, some mildly toxic, some harmless, and some useful, yet all possess electric mobilities which are, in magnitude, not far removed from those of pneumococci. While the virulence of one species of pathogenic bacteria is possibly directly proportional to the cataphoretic potential, the mere possession of such a potential does not necessarily make a bacterium toxic. What it probably does, if at all effective, is to make the bacterium more active; and if the bacterium happens to be a pathogen, then its greater activity, due to charge, makes it more deadly.

R. L. Thompson finds the same order of decreasing mobility of pneumococci (3, 1, 2) as does Falk, but he is unable to correlate this with the virulence of the bacteria.

The Migration of Leucocytes.—It has long been known that white blood cells (leucocytes) play an important part in wound healing. They are always to be found in great numbers in the vicinity of a wound. Their function appears to be primarily that of scavengers; they ingest bacteria present at the seat of the wound. They may also be agents in the production of wound-

healing secretions, or hormones. Our present question is, What lets the white blood cell "know" that it is needed at a certain locality? One possible interpretation is given by Abramson.

Differences in potential arise in tissues incidental to injury. The surface of a wound is negative on the outside and positive on the inside, as in the case of the apple (Fig. 154). Leucocytes are negative and may therefore be electrically attracted to the positively charged injured region. The deduction drawn is that the migration of leucocytes to an injured region is dependent upon electromotive forces present in the tissues. Any cell would tend to move toward the positively charged wounded area, but only the leucocytes can pass through the intervening tissue, for they possess the power of amoeboid movement. Their electric charge and that of the injured tissue merely indicate the direction of migration. (A fact worthy of note is that leucocytes behave like proteins in their cataphoretic properties, while red cells do not.)

Such theories are highly speculative but rest on experimental results. Obviously, they are not conclusive. Other factors may play a part. J. Comandon has illustrated, by moving pictures, that leucocytes will move toward a starch grain from quite a distance with extraordinary rapidity and in a remarkably straight path, pushing the red blood cells aside as they go. (Starch is insoluble in water; in the blood, it is broken down into sugars by enzymes; the sugars go into solution and probably act as the chemotactic substance.) Such a chemotactic attraction may, rather than an electrokinetic one, be responsible for the migration of leucocytes to a wound, or both may function.

Cataphoresis in Protoplasm.—The cataphoresis of microscopic granules suspended in the fluid protoplasm of cells was early recorded by Carlgren and others. C. V. Taylor has succeeded in demonstrating the cataphoretic migration of microscopic and ultramicroscopic granules in the fluid protoplasm of a slime mold. Dark-field illumination and an exceedingly weak direct current applied through especially designed, and very minute, non-polarizable microelectrodes were used. When the current was applied, the ultramicroscopic particles migrated, some to the anode and some to the cathode. A considerable group of the particles, still in rapid Brownian movement, remained in the center of the field and so were apparently electrically neutral.

Sen has observed the cataphoretic migration of particles in the protoplasm of the petiole hair of *Urtica* and the root hair of *Azolla* and finds them all to be negative.

Protoplasmic Streaming.—The streaming of protoplasm is a little understood phenomenon. Nearly all of the forces in the physical world have been resorted to in attempts to explain it! An electrokinetic interpretation is as reasonable as any other and has the advantage of some experimental evidence in its favor. The one-way protoplasmic streaming in the filaments of bread mold is possibly an example of electroendosmosis.. The protoplasm in the slender capillaries of bread mold flows many seconds, or even minutes, in one direction only and then returns. The first successful attempt experimentally to prove that such a protoplasmic flow is an electrokinetic phenomenon was made by Gelfan. He worked on the alga *Nitella* and was able to measure the potential set up between the two ends of the long cell by the streaming protoplasm. When the direction of streaming reversed, the direction of flow of current, as indicated by a delicate galvanometer, also reversed; and as streaming slowed down until the protoplasm was quiet, the voltage became less until it reached zero. We come to the inevitable, and usually unanswerable, question, Is the potential the cause or the result of the streaming? We cannot say, but it is of significance that a change in potential is associated with a change in direction of streaming. This Gelfan's work apparently proves.

Stability.—Electrokinetic properties of nonliving colloidal systems find application in the living world, usually without modification. Now and then, certain seeming exceptions arise. One of these is the stability of living particles. Joffe and Mudd report living bacteria in suspension with zero charge in the normal state. This may mean simply that potential as a stabilizing agent is here replaced by another, probably hydration. A water mantle is apparently in part responsible for the stability of protein suspensions (page 177). Bacteria, which, though in suspension, have no charge over the entire range of pH, probably have carbohydrate in their surface, to the hydration of which the suspension stability of the bacteria is due.

Northrop and deKruif tell of an interesting fact which is well known in nonliving colloidal systems. They have shown that a low concentration of salt precipitates bacteria, while higher

concentrations stabilize them. This may be due to the fact that excess salt or acid confers a high potential of opposite sign to that in low concentration, or the excess nutritive salt may depress the cohesive force between the colliding bacteria. The effectiveness of an electrolyte in reversing the charge of bacteria depends on the nature of the suspension. Thus, sodium chloride and sodium sulphate reverse the charge on the bacillus of rabbit septicemia but will only reduce the charge on *Bacterium typhosum*.

While the work of Joffe and Mudd minimizes the role of potential in stabilizing certain bacteria in general, it is yet true that most bacteria agglutinate when the potential falls to less than 15 mv. Northrop and Freund find that sheep-blood cells are not agglutinated by electrolytes until the potential is depressed to 6 mv. (except in the case of magnesium chloride and calcium chloride).

Other examples of agglutination—the reverse of stability—some of which rest upon electric forces, are considered under coagulation (page 479).

Kinship.—The discovery of the physiologist Landsteiner that there are four distinct types of blood in man and that these types can be determined by coagulation tests (page 503) led to the thought that there might be differences in rate of cataphoretic migration of the red cells. This did not prove to be true.

Work that had shown a relationship to exist between the blood of man and that of lower animals (apes and chimpanzees), as determined by coagulation, led Mez to ascertain if there was a protein relationship between plants. He found that there was (page 504). These findings led L. Moyer to seek another property which might indicate species relationship, *viz.*, the isoelectric points of particles suspended in the latex of rubber-bearing plants. About the same time Svedberg discovered a similar basis of kinship (page 505).

Moyer studied the electric mobilities of the latex particles of Euphorbias. *Hevea brasiliensis*, which is the commercial source of rubber, is not available in the greenhouses of temperate regions, but an excellent substitute is to be found among those plants known as the spurge which are species of Euphorbia. When a stem of Euphorbia is cut, it exudes latex. Latex is a fine suspension of hydrocarbon (rubber) in an aqueous medium of salts, carbohydrates, and proteins (pages 135, 385). It can be

readily withdrawn by tapping. The minute (0.5μ) globules of rubber remain in suspension and form an ideal material for the observation of the rate of migration in an electric field. In the discussion of emulsions and colloids in general, it was pointed out that the interphase, or that layer which coats the colloidal particle and separates it from the surrounding medium, is the material, or condition, that determines the properties of the

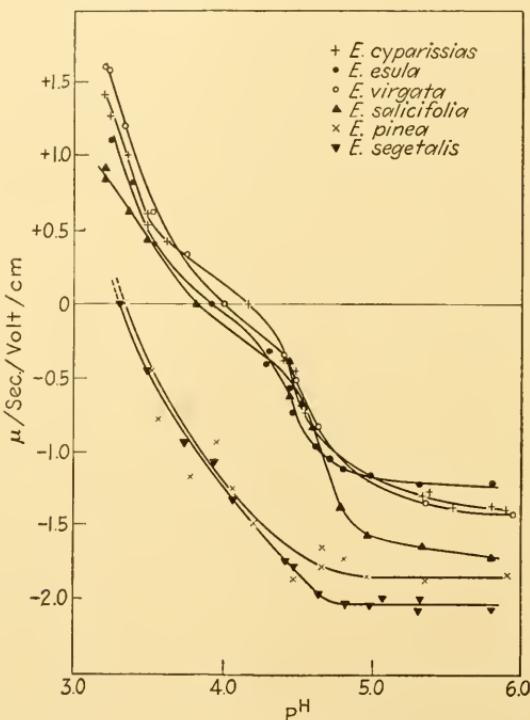


FIG. 165A.—Mobility curves of latex particles of *Euphorbia*. (From L. Moyer.)

colloidal state. So it is with latex particles; their rate of migration under the influence of an electric field, and their isoelectric point, will be determined not (primarily) by the rubber of which the particles are made but by the protein or other substance that coats them. If the surface layer is protein, it may show the same specificity as to species as do the plant proteins with which Mez worked and the proteins in blood. Moyer first established migration curves, plotting rate of mobility against acidity (Fig. 165A). The latex when collected is of a fairly constant pH

value; this can be changed to the acid or alkaline side by the use of buffers. The curves of a number of species of *Euphorbia* show a remarkable relationship. Those species known to be taxonomically related (as established by the time-honored methods of plant classification) yielded cataphoretic migration curves of identical form which crossed the line of no migration at almost precisely the same pH value, *i.e.*, they had the same

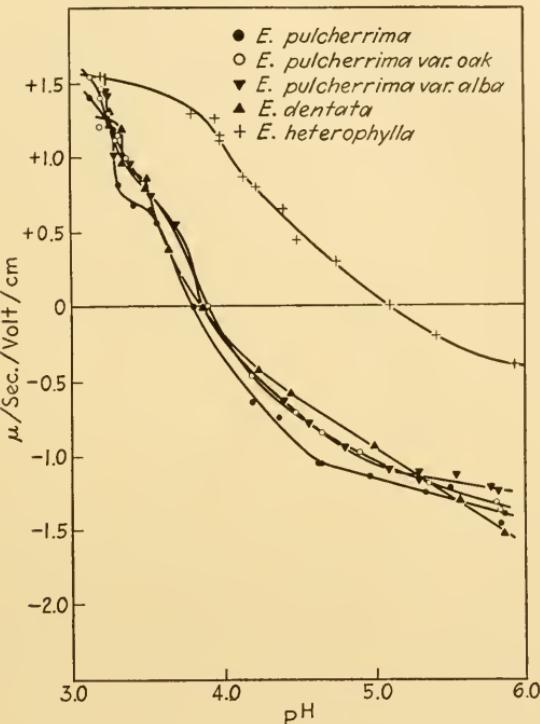


FIG. 165B.—(Continued.)

isoelectric points; while those species taxonomically not closely related yielded curves of different form, with other isoelectric points. An occasional species, which did not fall into any one group, proved to be an isolated form of questionable relationship on taxonomic grounds. Further study brought other interesting facts to light. The geographic distribution of the species was such as to agree with the protein relationship, and chromosome numbers showed similar conformity (though here the problem is a more involved one). If we analyze a group of curves (Fig. 165A), we find that the closely related species *Euphorbia*

cyparissias, *E. esula*, *E. virgata*, and *E. salicifolia* all give migration curves of like form and have isoelectric points all very close to pH 4.0. A second group (Fig. 165A) including *E. pinea* and *E. segetalis* showed the same relationship among its members, with curves and isoelectric points (pH 3.3) alike among themselves but different from those of the first group. The direction of migration of all species examined was readily reversed; *i.e.*, the original sign (negative) of the charge on the particle is reversed at the isoelectric point as stated in terms of a pH value. The rate of migration at a definite pH value, the degree of acidity at which the direction of migration changes (the isoelectric point), the shape of the curves, and the relationships established on these grounds all depend upon the nature of the substance that coats the latex particles. This coating appears to be protein, at least in some cases. In others, it is doubtful if proteins are the cause of the behavior. The question as to whether the specificity of the curves is due to specific chemical differences in the proteins or to different proportions of the same proteins composing the mixture on the surface is as yet undecided. The latter surmise appears to be the more correct one. In other words, whatever the coating substances may be—proteins, alcohols, fatty acids, or carbohydrates—in each taxonomic group of species there seems to be a definite mixture composing the surface of the latex, different from that of any other species. Mez appears to be dealing with *qualitative* differences in proteins shown by reactions of families with each other but giving no differences in reaction from species to species. Moyer may be showing a *quantitative* difference which is also specific. We find, therefore, that there is a definite relationship between the coatings of latex particles from different species of plants which is in agreement with the taxonomic relationship of the plants.

The geographic distribution of the species is also in keeping with this relationship. Thus, *E. virgata*, *E. cyparissias*, *E. esula*, and *E. salicifolia* all come from central and southern Europe and are closely related. Of the four, *E. salicifolia* diverges most widely in distribution and also in the curve shape. Both *E. pinea* and *E. segetalis*, with like curves and like isoelectric points, come from the Mediterranean region.

Chromosome counts show an agreement between species which harmonizes quite well with latex, taxonomic, and geo-

graphic relationships. In Fig. 165B, the curves of two species and two varieties which are known to be of close relationship; all four have similar migration curves and practically identical isoelectric points; each was found to have 28 chromosomes. *E. heterophylla* (Fig. 165B) was shown by its latex behavior to be an isolated species; it was found to have 56 chromosomes.

The discoveries of Landsteiner, Mez, Moyer, and Svedberg are among those pioneer contributions in which biological phenomena, heretofore little understood, are shown to rest upon readily determinable physical and chemical properties. Mobility curves and isoelectric points are not the recognized bases of species classification, but they indicate kinship as much as do flower structure, facial characteristics, and like criteria of racial grouping.

CHAPTER XX

RADIANT ENERGY

Man has long been aware of his radiation environment. He has known that he is sensitive to the rays of the sun, that they warm him, tan his skin, and improve his health. He has also suspected that moonlight, which differs from sunlight, has its influence, though here his imagination has had the best of him at times. Within recent years, science has substantiated many of these beliefs and added others of which primitive man never dreamed, for our radiation environment is by no means limited to light as we ordinarily think of it. No one with the slightest scientific knowledge would today question the possibility of organisms, and therefore protoplasm, being sensitive to such forms of radiation as ultraviolet light, electric rays, X rays, and cosmic radiation. It becomes our task to determine how far living matter responds to its radiation environment.

We have not only the problem of the effect of external radiation on protoplasm but also the possibility of protoplasm being itself a source of radiation. This question takes us into a much controverted field; but if we consider the problem subjectively, we are forced to come to the conclusion stated by F. Daniels, *viz.*, that there is no fundamental reason why protoplasm should not give off photons, or units of light energy.

The Electromagnetic Spectrum.—Forms of radiant energy now known include electric (hertzian or radio) rays, heat, infrared, visible light, ultraviolet, X rays, gamma rays, and cosmic rays. All can be placed in a scale of wave lengths extending from waves several miles in length to waves so small that we cannot appreciate their minuteness (Fig. 166). It is extraordinary that these numerous and diverse forms of energy are all waves, apparently just alike except for length, and yet how different they seem to us when we consider them separately.

The discovery of the essential unity in such seemingly different forms of energy as occur in the *spectrum of electromagnetic radi-*

ation is one of the greatest achievements of modern science and was anticipated by Faraday. The name given to the spectrum implies that electricity, magnetism, and radiation (light) are one. Faraday went further, for he had hopes of coupling such physical forces as electricity (voltaic and static), gravity, heat, cohesion, and magnetism. He discovered the principle of electromagnetic induction, and we now speak of the principles of electromagnetism. He saw the connection between magnetism and light, and we now speak of magneto optics. He realized the bearing of electricity upon chemistry, and we now have electrochemistry.

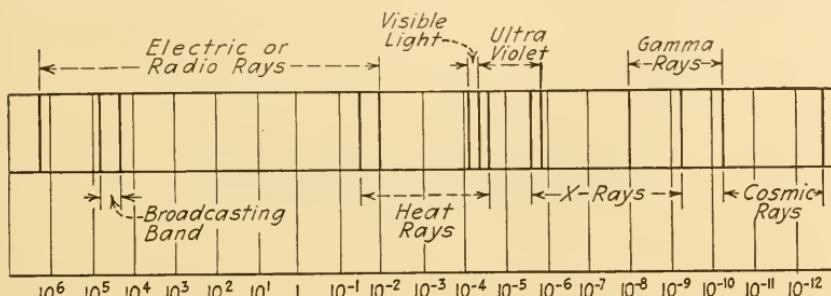


FIG. 166.—The spectrum of electromagnetic radiation (wave lengths are in centimeters on a logarithmic scale).

Let us briefly consider the electromagnetic spectrum (Fig. 166), starting with the hertzian waves of longest length. These electric or radio waves undoubtedly affect living matter, but we know little of their influence. The somewhat shorter heat waves constitute a world of their own and will not be considered here. Their effect is known to all in a general way.

Visible light comes next; it appears to be homogeneous, but actually it is made up of many colors which can be separated by passing white light through a glass prism. Composite white light is diffracted into the infinite colors of the spectrum of which violet, indigo, blue, green, yellow, orange, red are characteristic. The longest visible rays are 7,500 A. U. (0.75μ) in length; the central yellow ones are 6,000 A. U.; and the shortest visible violet ones, 3,900 A. U. long. Many systems, living and nonliving, are selectively sensitive to the rays of the spectrum. Most photographic negatives are sensitive to the blue end and practically insensitive to the red. Certain negatives are now made sensitive to the red end of the spectrum. Light rays of

different wave length produce different effects on human beings, especially when the rays lie beyond the two ends of the visible spectrum. The actinic qualities of the ultraviolet are much greater, as measured by their influence on human skin, than are those of the infrared. It is the ultraviolet which tans and blisters the skin. Ultraviolet light extends from the violet end of the visible spectrum at 3,900 A. U. to below 1,000 A. U. Beyond, but overlapping the ultraviolet slightly, lie the X rays.

Just as the wave lengths of the infrared rays ultimately become, upon a gradual increase in length, those of radio rays, so do those of the ultraviolet, on a decrease in length, become X rays. When shortest—between 1 and 0.1 A. U. in length—X rays are

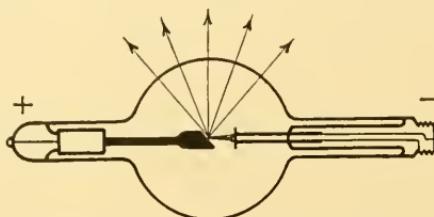


FIG. 167.—The Crookes or X-ray tube.

most penetrating and are then termed "hard." At this length, they become gamma rays, the usual source of which is the disintegration of atomic nuclei. X rays were discovered by Roentgen, by whose name they are also known. X rays are produced in a cathode tube and come into existence when cathode rays strike the anticathode, the anode itself, or any other surface, as the result of high electrical tension between the cathode and anode poles of the tube. The original laboratory source of X rays was the Crookes tube (Fig. 167), which has now developed into the Coolidge tube, a more compact and much more efficient type, wherein other forms of radiation have been eliminated to a great extent.

As in the case of ultraviolet light, X rays do not make an impression upon our eyes, but if a fluorescent screen of platinum barium cyanide is placed in their path, a brilliant glow results. The same effect is obtained when invisible ultraviolet strikes a fluorescent screen. This similarity in the behavior of ultraviolet and X rays and the fact that both affect the photographic plate indicate that X rays are identical with or of the nature of light rays, yet when first studied they could not be diffracted or scat-

tered as can light. When a wave front strikes a grid, such as a piece of glass upon which many close parallel lines have been etched, the wave is broken up into smaller waves (Fig. 168). The wavelets thus formed combine to form a new wave front. The same diffraction effects can be obtained with sound waves of much greater length. (Diffracted sound waves have been photographed.) If X rays are of the nature of light rays, it should be possible to diffract them. The German physicist von Laue had the brilliant thought of using the symmetrical distribution of atoms in a crystal (Fig. 143) as a superfine grid with which to diffract X rays. The experiment was successful and opened up an entirely new and highly satisfactory method of studying

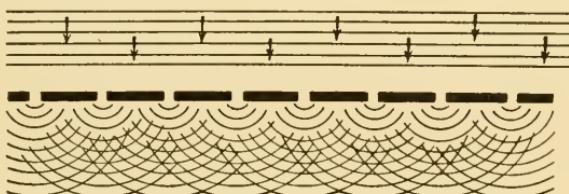


FIG. 168.—The diffraction of light by a grating.

crystal structure, which has been carried so far and so successfully by William Bragg.

Other types of radiation produced by a vacuum tube include cathode rays, or negatively charged electrons, and canal rays, or positively charged ions. Cathode rays are negative electrons and come from the cathode. Canal rays were so called because in order to observe them, it was necessary to drill a hole in the metal of the cathode through which some of the canal rays found their way. Canal rays appear to emanate from the anode but actually do not do so; instead, they originate in the tube, somewhere in front of the cathode, as a result of ionization of the gas; being ions, they are obviously much heavier than the electrons of cathode rays. Anode rays are another type of radiation from a vacuum tube which may be regarded as distinct from canal rays, though perhaps at times at least they are the same. They are seldom referred to, because as a distinct type they are not evident in an ordinary vacuum tube with simple metal electrodes; they may be produced by coating the anode with a metallic salt which disintegrates under an electric charge across the tube and gives forth positive or anode rays. Biologically, the X rays of a

vacuum tube are of particular interest, though undoubtedly the other forms of emanation from such tubes have their effect on organisms. Cathode rays have already been used in biological experiments.

Passing on toward that end of the spectrum with shorter waves, we come to the gamma rays which overlap the X rays. Gamma rays are emitted by radium. They are more penetrating than X rays and have the property, like the latter, of ionizing (charging) air. Gamma rays will penetrate a foot of water and still have half of their intensity left, while X rays are absorbed by an inch of water.

The wave spectrum ends with cosmic rays, the shortest known—so short that were they increased in length until they equaled the thickness of a card—then the longest wireless wave, if enlarged in the same proportion, would extend from the earth to the nearest star. Cosmic rays have come into considerable prominence of late. Their presence in the atmosphere was first suspected by Rutherford. He thought them due to the radioactive materials in the earth. At that time, they were referred to as the “penetrating radiation” of the atmosphere. When it was found that this radiation did not decrease so rapidly as it should with altitude, the German physicist Gockel took an electroscope up in a balloon to a height of 13,000 ft. and found that the penetrating radiation still did not decrease. Higher altitudes (5.6 miles) revealed a radiation several times as great as that at the earth’s surface, which indicated that the radiation must be of cosmic origin. Millikan then continued the work and measured the intensity of the rays in a mountain lake to a depth of 45 ft. The atmosphere above the lake was equivalent in absorbing power to 23 ft. of water, so that cosmic radiation can penetrate 45 plus 23 or 68 ft. of water, the equivalent of 6 ft. of lead. Cosmic rays are “harder,” more penetrating, than any others known.

Some interesting speculation has been done on the possibility that the source of cosmic rays is the energy lost when hydrogen unites to form helium. Millikan regarded the presence of cosmic radiation as direct evidence of atom building out in the depths of interstellar space. The atomic weight of hydrogen is 1.0078; the mass of the helium atom is 4; therefore, when four hydrogen atoms unite to form one helium atom, there is a loss

of energy equal to 4×0.0078 , or 0.0312. That hydrogen is not a whole integer, as the law of exact multiples among atoms would require, is to be explained by the fact that hydrogen is the only atom in which the nucleus does not consist of a close packing of electrons. With close packing, there is a reduction in effective mass; therefore, energy must be released whenever free electrons (negative and positive electrons or hydrogen nuclei) unite to form atoms. The free proton has a mass of 1.0078, but the combined proton in the molecular state has a mass of 1. This loss of 0.0078 unit of energy has been presumed to contribute to cosmic radiation. Such a speculation is supported by the philosophic argument that if the heavier elements are disintegrating into lighter ones, as are, for example, rubidium, thorium, and others into lead, then there probably exists, somewhere, a building-up process taking place to compensate for the breaking-down process; as Millikan says, cosmic rays constitute the announcement from the heavens of the birth of elements. The speculation is an interesting one and has something in its favor, but there is insufficient evidence to support it, and certain experimental facts are against it. T. H. Johnson has shown that much cosmic radiation consists of positively charged particles; this tends to disprove the atom-building hypothesis. There is also the unlikelihood of a chance collision of atomic (hydrogen, helium, or other) nuclei taking place in a gaseous medium as rare as is interstellar space. If the union of the 26 atomic nuclei necessary to form an atom of iron should take place but once, the union of 4 hydrogen nuclei to form one atom of helium would be enormous, and there is no evidence of such quantities of helium. Whatever their source, cosmic rays are real and are now regarded as positively electrified particles (because they are affected by the earth's magnetic field), shot toward the earth from remote distances at nearly the speed of light. That they are waves is another possible view.

We have so far referred to radiant energy as a manifestation of waves, just as if there were no doubt about it. The fact that many kinds of energy (*e.g.*, forms of light) consist of waves is presumably proved by their capacity to be bent and scattered as in the phenomena of diffraction and interference. The fact that we measure the wave length of light is tacit admission of its wave character. But Newton thought differently about it.

He defended the view that light consists of streams of minute particles. He believed this because light travels in a straight line which is characteristic of moving bodies. His contemporary, the Dutch physicist Huygens, advocated the wave theory (1678) and introduced the concept of an all-pervading ether to explain the transmission of the waves through space. So great was Newton's influence that the only prominent men of the eighteenth century who supported the undulatory theory were Leonard Euler and Benjamin Franklin. But during the next century, things were reversed, and the corpuscular theory of Newton fell into ill repute—so much so that, fifty years ago, S. P. Langley wrote of it as "pernicious," for, if light is material, then "radian heat, if affiliated to light, must be regarded as material too." And now, in the twentieth century, both theories are accepted.

The first break from the wave theory of light was initiated by the German physicist Planck, who stated that when a body becomes red hot, it does not give off energy continuously, as the wave theory would demand, but radiates heat in discontinuous units, bursts, or *quanta* of energy. Einstein then called attention to the fact that Planck's conclusion would be satisfied by the view that radiation is not wavelike, but is of matter, that is to say, consists of particles. Thus arose the quantum theory which in certain features resembles Newton's corpuscular hypothesis. The particles, or quanta, of light are now known as *photons*. The energy value of a quantum is the product of the wave frequency v and the constant of Planck h . Expressed in this way, the energy of a quantum of green light corresponds to 2.5 electron volts. On the same basis, the energy given off when four hydrogen atoms unite to form one helium atom is 27,000,000 volts.

The diffraction of light, and of X rays by crystals, is convincing evidence that light and X rays consist of rays. The photoelectric effect is just as convincing evidence that they consist of particles. If light falls upon metals such as zinc or sodium, a current of negative electricity—a stream of electrons—escapes from the metallic surface. This is the photoelectric effect. It is more prominent in the case of X rays, which have the capacity to eject electrons from many kinds of surfaces. Physicists saw the futility of trying to explain the photoelectric effect on the basis of waves. Only particles—photons—can account for it.

The apparent dual nature of light is best viewed not as such but rather in terms of the philosophy of *as if* (a concept frequently employed in physics and applicable to such expressions as "curved space"). Light behaves *as if* it were wavelike in character. Diffraction phenomena prove this. Yet it is certainly corpuscular in the sense that it can strike a single electron and give up all of its energy and momentum to that electron (as in the photoelectric effect). In other words, the particle moves as if it were a wave; but when it collides, it *behaves* like a particle, *i.e.*, it has mass as well as energy. We may now transfer the discussion of the particle *vs.* the wave theory of matter from the photon (light) to the electron.

The first constructive hypothesis which led directly to modern theories of atomic structure was that of the English physicist Ernest Rutherford. He, in 1911, conceived of the atom as a miniature replica of our solar system. The "sun," or nucleus of the atomic "solar" system, was presumed to be a compact cluster of positively charged particles, and the surrounding "planets" were negative *electrons*. (It is said that the Japanese Nagaoka, in 1904, independently advanced the same idea, but credit is given to Rutherford, for he supported the theory with experimental facts.) Such a postulate was not possible without the experimental work that preceded it—the discovery of X rays by Roentgen (1895), of the radioactivity of uranium by Becquerel (1896), the recognition by J. J. Thomson of the electron as the unit of electricity (1897), and the conception of energy quanta by Planck (1900). Then followed much speculation on the arrangement of the positive and negative electrons and on the energy relationships within the atom. G. N. Lewis and Irving Langmuir advocated a static atom; that is to say, they assumed that the electrons, or planets, are stationary or move only within very restricted regions. The Danish physicist Niels Bohr took the opposite point of view and regarded the atom as a dynamic system in which the electrons travel in definite orbits around the nucleus, just as do the planets of our universe around their sun. Chemists at first rather preferred the static atom; it fitted in better with their ideas of valence. If atoms unite by sharing an electron, it is easier to visualize their doing so if the electron is relatively quiet rather than traveling at terrific speed in an orbit. Objection was also raised on the ground that the electrons

would eventually run down, and then, too, why this tremendous waste of energy? But one of the postulates of the Bohr theory is that no energy is spent (except during radiative transitions). The dynamic atom was preferred by physicists and is now generally accepted.

According to the theory of Bohr, as amplified by modern concepts, hydrogen, the lightest and simplest element, contains



FIG. 169.—Diagram of the hydrogen atom.

a central nucleus consisting of a *proton*, which is one elemental particle, or *neutron*, plus one positive electrical charge, or *positron* (Fig. 169). Outside this, revolving in an orbit, is a planet of one negative charge—the negative *electron*, or *negatron* (the negative electron is presumably the larger of the two, but the proton is much the heavier, being nearly two thousand times the weight of the former). Thus is the essential mass of the atom centered in the nucleus. The distance between the “sun” and “planet” is very great in proportion to their sizes, just as in our solar system, which leads to the startling conclusion that the atom, and consequently all matter, is mostly space.

To account for certain energy relationships, Bohr carried his concept of atomic structure still further. He assumed that the

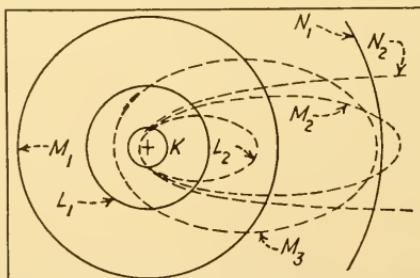


FIG. 170.—Diagram of the various possible orbits, *K*, *L*, *M*, *N*, of the hydrogen atom.

lone negative electron of hydrogen has an inner orbit where it normally revolves but that it may travel in several other outer orbits to which it jumps and stays a while, though its natural tendency is to migrate back to the innermost orbit (Fig. 170). The several orbits or energy levels in which the electron may travel have been lettered *K*, *L*, *M*, etc. Owing to an attraction between the planetary electron and the nucleus (due to elec-

trical rather than gravitational forces, as in the case of our earth and sun), the electron tends to occupy the innermost orbit *K*. The radius of this orbit is that of the hydrogen atom. In going from one orbit to another, the electron takes on energy when passing outward and gives it off when passing inward, to the amount of one quantum, or unit of energy, when jumping from one orbit to another. This capacity of the electron to give off explosive bursts, or quanta, of energy in going from one orbit, or energy level, to another is the source of Roentgen rays.

The second element in the periodic table is helium. Its atomic number is 2, and its weight is 4. Helium has, therefore, two planetary electrons in the outer field and four neutrons in the nucleus, two of which possess positive charges (*i.e.*, are protons) and two of which are of zero charge (Fig. 171). This constitution of atomic nuclei is characteristic of all elements except hydrogen, *viz.*, enough neutrons to account for the atomic weight and enough positive charges to balance the outer field electrically.

Each succeeding element of increased weight in the periodic table has one additional electron in the outer field balanced by an additional positive charge in the nucleus, with enough neutrons to account for the increase in weight. For example, a moderately heavy element, such as calcium, with atomic number 20 and atomic weight 40, possesses 20 outer electrons and a corresponding number of protons, with 20 additional neutrons in the nucleus.

Having at our disposal all the elementary units necessary to construct matter, it will be instructive to list them in the table shown on page 400.

One additional fact is necessary to complete the picture of atomic structure and to emphasize the importance of the energy relationships resident there. It also indicates how closely atomic structure duplicates, in plan, the arrangement of bodies in our solar system. Goudsmit and Uhlenbeck are given credit for

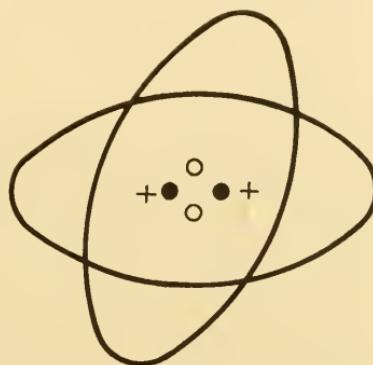


FIG. 171.—Diagram of the helium atom.

the hypothesis that electrons, like planets, are not only traveling their celestial orbits but spinning on their own axes.

Name	Charge, e.s.u.	Mass
Negative electron, or negatron.....	$-e = -4.77 \times 10^{-10}$	10^{-27} grams
Positive electron, or positron.....	$+e = 4.77 \times 10^{-10}$	10^{-27} grams
Neutron.....	0	1.8×10^{-24} grams
Proton, or hydrogen nucleus.....	$+e = +4.77 \times 10^{-10}$	1.8×10^{-24} grams
Alpha particle, or helium nucleus.....	$+2e = +2 \times 4.77 \times 10^{-10}$	$4 \times$ proton
Photon, light quantum, X-ray quantum, or γ -ray quantum.....	0	0

Newer Theories of Atomic Structure.—Modern theories which substantially modify, indeed in the main replace, the older (Bohr) theory of atomic structure, have been advanced by de Broglie, Heisenberg, and Schrödinger. Important as these newer theories are, it is still true, as Ruark and Urey state, that “the conception of a planetary atom, governed by ordinary mechanics, will remain a useful tool for many years, whatever theoretical developments may be.”

The fundamental concepts of the new physics are the substitution of quantum mechanics (the wave theory) for the older Newtonian mechanics and the substitution of probability for certainty. It had its beginning in the wave theory of motion advanced by the French physicist Louis de Broglie. As in the case of light, physicists found that some phenomena are more easily interpreted if the electron is considered to be a particle, while others are better explained if the electron is regarded as a wave. De Broglie, therefore, suggested that if light radiation has the properties of both waves and particles, this might also be true of electrons; that is to say, electrons are corpuscular, yet they move through space by mechanical laws which are not the same as Newtonian mechanics, and this motion can be best represented as the motion of waves.

The newer physics has gone still further. It tells us that when ideas have to do with things that approach the infinite, they perforce become less and less definite. Imponderables, incom-

prehensibles, such as the dimensions of atoms and the velocities of electrons, force one to resort to probability summations; for example, if the diameter of an electronic orbit is of the order of 10^{-8} (0.00000001) cm., and electronic velocities approximate 10^9 cm. (100,000,000 cm. = 1,000 km.) per second, then the time for an electron to make an average revolution would be of the order of 10^{-17} (0.0000000000000001) sec.—a period so short that it cannot be separately considered experimentally. If we think in terms of ordinary time and space, then an electron is everywhere at once, remaining only within the confines of its shell. Rather than attempt to give a comprehensible picture of such an atom, the newer quantum mechanics gives only a probability—that of finding the electron in any specified region at any one time.

The probability, or uncertainty, principle of Heisenberg, based on the concept that the precise path or position of an electron at any one moment is not predictable, led him to state that there must be a renunciation of old and cherished ideas, such as the principle of causality or the belief that natural phenomena always obey exact laws. In support of Heisenberg's statement, A. H. Compton points out that if the principle of causality is replaced by that of uncertainty, all those paradoxes of atomic physics that have been classed as "quantum phenomena" find a ready solution. This includes the emission of light, the photoelectric effect, etc. (It should be remembered that classical laws when applied to large-scale phenomena need not be seriously affected by the principle of uncertainty; in other words, the macroscopic world is predictable, but the microscopic—the atomic—world is said not to be.)

While the uncertainty principle—the principle that one cannot predict a future position of the electron on the basis of an initial position and velocity, primarily because the present circumstances are partially unknowable—is now widely accepted by physicists, there are yet those who oppose it, both as a philosophical idea and as a mathematical deduction, and maintain that the physical sciences are still based on the strict and universal validity of the principle of causality.

An attempt to formulate a picture of the atom in terms of probabilities has been made by H. E. White. Photographs of a small mechanical model are made which represent pictures that

one would expect to get if the electron could be photographed. From Fig. 172, it is seen that there are certain preferred regions for the electron, *i.e.*, regions where the probability of finding the electron is relatively large, and unpreferred regions.

The old and the new pictures of the atom are quite different in their formulations and in their energy relations, except for hydrogen. Here the old Bohr orbital picture and the new quantum mechanical one give exactly the same energy relations for the atom.

With the foregoing as a basis of discussion, we may now consider the forms of radiant energy given off by atoms. There is first the energy emitted when an electron changes its orbit, from an "excited" to a normal state. If we take the simple case of hydrogen, we shall remember that its one electron can have a

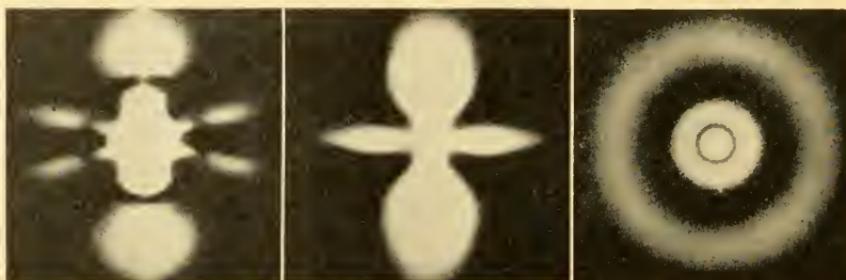


FIG. 172.—Photographs of the electron cloud for three states of the hydrogen atom, obtained from a working model. The cloud shows where the electron of hydrogen may be at any given moment. (From H. E. White.)

number of possible orbits, in each of which it possesses a definite energy value calculable in terms of the radius of the orbit, according to the same principles for the calculation of the energy associated with the rotation of a planet around the sun. The greater the size of the orbit the greater the energy. Only by acquiring energy can an electron be made to leave its orbit and go into a larger one. In returning from a larger orbit to a smaller one, it parts with this energy, which is radiated in the form of light or X rays.

Other types of energy radiated by an atom are alpha and beta particles and gamma rays. Alpha particles are the nuclei of helium atoms and therefore consist of two neutrons and two protons (*i.e.*, four neutrons, two of which have positive charges). Alpha particles are, therefore, helium atoms which have lost

their two outer electrons and thus possess a positive charge of $2e(2 \times 4.77 \times 10^{-10}$ e.s.u.). They are very easily absorbed, being completely stopped by 0.1 mm. of aluminum or a few centimeters of air.

Beta particles are free electrons and therefore carry a negative charge. They move at high speed, being almost as rapid as light rays, and are more penetrating than alpha particles passing through a millimeter of aluminum (or a meter of air). They are identical with cathode rays, differing only in velocity.

Gamma rays are regarded as pulses in the ether of very short wave length (about 10^{-9} cm.), just as are X rays and all other light rays (Fig. 166). Gamma rays, X rays, and ultraviolet rays may be grouped together because of similar characteristics. Gamma rays are exceedingly penetrating, those from radium producing measurable effects through a thickness of 20 or 30 cm. of iron. Both beta and gamma radiation is given off not only by the heavy radioactive elements but also by lighter elements such as potassium, which suggests that potassium is transmuted into calcium.

Alpha, beta, and gamma radiation is given off by radium and causes it to degenerate ultimately into lead. (It requires about 20,000 years for the degeneration of radium and more than 4,000,000,000 years for uranium to become converted into lead.) There are 88 positive charges (balanced by negative planetary electrons) in a radium atom and 92 in a uranium atom. If but six of these are to be lost over so great a period of time in order to produce an atom of lead (atomic number 82), then the number likely to come from one atom in a given interval of time must be very, very small indeed. That we observe a constant stream of electrons coming from a piece of radium is due to the fact that the piece contains billions upon billions of atoms. (10^{15} , or a thousand million million atoms, are in 4×10^{-7} , or four ten-millionths of a gram of uranium.)

There is an interesting feature of radium radiation which is in keeping with the uncertainty principle of Heisenberg. The rate at which an infinitely small amount of radium will radiate is not known with certainty; that is to say, if the amount is reduced to one atom, the frequency with which it will give off a particle is not predictable. Furthermore, the event takes place within the minute universe of the atom with as much likelihood as that our

sun should throw off a large part of itself or that Mars should suddenly change its orbit.

There are a number of types of secondary radiations due to the primary types; thus, when ultraviolet light and X rays fall upon a metal, they eject negative electrons. This emission of electrons under the influence of light is called the photoelectric effect. (It has been mentioned that the phenomenon is difficult to account for on the wave theory of light but is readily interpreted in terms of the corpuscular theory.)

IN THE LIVING WORLD

Having acquired some knowledge of electromagnetic and atomic radiation, we may consider the effect that these forms of radiation have upon living matter. There is both experimental evidence and the evidence that comes from daily experience to show that organisms are at times very sensitive and at other times very resistant to their radiation environment. Man's knowledge of his radiation environment came first through his familiarity with the influence of the sun's rays. Solar radiation is intermediate in position in the spectrum; it is quite likely, therefore, that those parts of the electromagnetic spectrum beyond visible light will also have an effect on living matter.

Visible Light.—The influence of visible light on life is so great that we can select only a few of the less well-known examples for consideration. Solar radiation warms the earth and the organisms on it. It is the energy by means of which plants carry on that primary chemical reaction upon which nearly all forms of life depend, *viz.*, photosynthesis. Plant stems and leaves usually turn toward the light; plant roots and many lower forms of animals turn from it. Light determines the rate of growth of plants. Dandelions grow to the height of the surrounding grass; if the grass is cut, the flowers are formed on shorter and shorter stems. The shape of trees is often determined by the stopping of the growth of the limbs by light. The very symmetrical form of some fir trees is due to the influence of light.

The growth rhythm, so typical of organisms in general, might very likely, in the case of plants, be attributable to temperature; thus, seeds sprout and buds open in the spring because of warmth, while plants die and leaves fall in the autumn because of frost. Temperature is undoubtedly a factor. But Garner and Allard

have made the interesting discovery that certainly the time of flowering if not the actual flowering of plants is, within limits, determined by the length of daylight to which the plants are exposed. They were able, by controlling the time of exposure of a plant to light, greatly to decrease or increase the age at which the plant reaches sexual maturity. Thus, the field aster, which commonly requires four months (May to September) to reach sexual maturity, was made, by decreasing the time of exposure to daylight, to bear flowers within a month after germination (by June 18). Still more remarkable is the fact that these same plants, instead of completing their life cycle by dying after flowering, as they would have done in the field, developed new axillary branches (on being restored to normal light exposure) and flowered a second time in September.

It is thus evident that certain characters of a deep-seated and fundamental nature, which heretofore have been regarded as immutable, are relatively unstable and respond readily to change in the external environment. It is, consequently, not surprising for some biologists to hold that all "characters are of the nature of responses to environment" and that "every life process must to some degree be dependent upon the external world."

These studies assume a practical significance in connection with the growing of plants in North Russia and Alaska where the season is short and daylight limited. If plants can be forced into flower and fruit more quickly by shorter or longer exposure to light, then it will be possible to bring fresh fruit and vegetables on to the markets at a time that under normal conditions would not be possible.

The question of the relative significance of the different parts of the spectrum on photosynthetic activity has long existed among botanists. The importance of ultraviolet light was at first emphasized, until it was realized that plants do well in greenhouses the glass of which permits little or no ultraviolet to enter. Emphasis was then laid on the infrared. It now appears that plants can use both ends of the spectrum—that while the blue-violet (and ultraviolet) end of the spectrum is not indispensable, it is necessary for vigorous growth. Absence of all wave lengths shorter than $529\text{ m}\mu$ results in a condition similar to that in which plants are grown in very subdued light; infrared-illuminated plants are larger but less green. The infrared is, therefore,

not sufficient for normal growth. Funke finds that in blue light, plants do as well as in normal light; while in red light alone, they become etiolated.

Polarized Light.—Organisms are accustomed to direct light. Only comparatively rarely are they subjected to reflected light. Reflected light is polarized. It has recently been found that polarized light has a marked effect on living and nonliving matter. This form of light consists of unilateral vibrations swinging parallel to each other in the same plane. Ordinary light vibrates in all planes; it is propagated from its source in all directions. The light from the moon is polarized because it is reflected. Our eyes cannot distinguish polarized from ordinary light, but living tissues and photochemical reactions apparently possess the capacity to distinguish between them. The work of David Macht and of Bhatnagar indicates that polarized light accelerates chemical reactions (between amalgams of the alkali metals); profoundly affects the properties of drugs; and stimulates the growth of bacteria, yeast, and seedlings. It seems that the first experiments of this nature, on the conversion of starch by polarized light, had been done earlier in England. That we shall yet have to grant the possibility of there being some truth in the old superstitions that moonlight is harmful—from which are derived such familiar expressions as “lunatic,” “moon blind,” and *mondsüchtig*—and that certain fruits are ripened primarily by the light of the moon seems unlikely, if for no other reason than that the light of the moon is very feeble; to have any effect, it would have to exert its influence over a long period of time.

Infrared.—At the long-wave end of the visible spectrum are the infrared rays. These have at last come into their own, the stage until now having been held by ultraviolet. Infrared rays play a very significant part in life—more so than we have heretofore realized. They are coming to the fore in physiotherapy for the treatment (“baking”) of strained muscles. In photography, also, infrared rays give pictures of greater depth and clarity on plates sensitive to them. Reference has already been made to the infrared as a source of energy for plants.

Ultraviolet.—Just beyond the short-wave end of the visible spectrum lie the ultraviolet rays. These have come to play so important a part in our daily lives that nearly everyone now knows of their necessity to our well-being. Perhaps the most

startling and valuable medical use of ultraviolet light is in the cure of rickets, a use that can be demonstrated by the following experiment. One young chicken is allowed to run under normal conditions out of doors, and two are kept within a greenhouse. The glass of the greenhouse does not permit ultraviolet light to penetrate. One of the chickens in the greenhouse receives the ultraviolet light of the sun or of a sun lamp for fifteen minutes each day. In time, it will be found that the chicken running free outside will be a normal one; so also the one in the greenhouse treated to ultraviolet light, while the untreated one in the greenhouse is a weak, undeveloped dwarf. The undeveloped chicken is a victim of rickets.

There are two cures for rickets—sunlight (ultraviolet) and cod-liver oil. It is possible to "bottle up" sunlight in a number of ways and make medical use of its beneficial effects. The greatest advance in the collecting of sunshine for medical use has been in the treatment of ergosterol with ultraviolet light. A highly efficient substance for the cure and prevention of rickets is thus produced (page 464). Whether it is ergosterol, milk, or another substance that is irradiated, the energy resident in ultraviolet light has caused certain changes in molecular orientation which impart curative powers to the treated substance. The antirachitic portion of the spectrum extends from 3,100 to 2,400 A. U. Because of poor adsorption by the skin, the extremes are not very effective. This is essentially the range of the adsorption spectrum of ergosterol.

Such a specialized use of ultraviolet rays is for the specialist to handle; we, who are laymen, can make good use of the health-giving powers of ultraviolet light by letting the rays of the sun play upon our bodies and penetrate every corner of our homes. As a disinfectant, ultraviolet light has hardly an equal. Excessive and intense ultraviolet radiation may cause considerable harm. The indiscriminate use of sun lamps is inadvisable.

The spectrum limits of ultraviolet light are from the visible at 3,900 to nearly 1,000 A. U. It is the longer part of this range, just beyond the visible region, which is most beneficial for plant and animal life. According to Withrow and Benedict, plants do best (grow tallest) in ultraviolet light of 2,970 (2,900 to 3,100) A. U. Very short wave lengths (shorter than 2,900 A. U.) are harmful to both plants and animals. Experimenters,

therefore, cannot use unfiltered ultraviolet light if their results are to be of significance. Results may be negative simply because one wave length is having an effect in one direction and another wave length is producing an effect in the opposite direction.

Studies of the effect of ultraviolet light on protoplasm are as yet in their infancy, but something has already been accomplished. Lepeschkin, in continuing his work on the effect of light on protoplasm wherein he found that visible light decreases the stability of protoplasm and its resistance to temperature, to mechanical injury, and to alcohol, observed that short exposures of ultraviolet rays increase the stability of protoplasm, while long or heavy exposures decrease it.

X Rays.—Beyond the ultraviolet lie the shorter X rays. They represent one of the most important forms of radiation in therapy. They are used extensively in the treatment and killing of organs and growths (cancer). By centering a beam of X rays on an organ within the body and irradiating for a short time, then rotating the body so that the organ to be treated or killed always receives the rays while the surrounding tissue is subjected to the rays but once, the organ becomes heavily radiated and may be killed without intermediate cells suffering from the treatment.

The therapeutic value of X rays, sunlight (ultraviolet), and gamma rays lies in their ability to strip electrons from the atoms upon which they fall—atoms such as those of sodium and calcium, which, as a result of their electronic loss, gain new combining powers. They are, therefore, able to enter into new chemical combinations and form new compounds which get into the blood stream and there act as curative agents. It is an encouraging sign in the progress of science when so little understood a process as the therapeutic value of radiation can be interpreted in terms of so fundamental a physical theory as that of atomic structure.

X rays, more than ultraviolet, have their dangers from excessive or misdirected use.

The effect of X rays on protoplasm is in the early stages of experimentation. Nadson and Rocklin find the first noticeable effect to be an acceleration of streaming. Stimulation is followed by depression. In yeast, there is an increase in the number of fat droplets. Permeability changes also occur. The latter

are typical degeneration effects likely to occur in any dying cell. Williams finds similar effects with both X rays and gamma rays. While pronounced changes usually occur, Weber finds great resistance; for example, there is no change in the viscosity of the protoplasm of *Spirogyra* and of *Phaseolus* seedlings with treatment—which merely goes to show that protoplasm is not everywhere the same.

The effect of X rays on developing eggs may appear some time after treatment, a phenomenon not uncommon in medical radiotherapy, where the secondary effects of radiation are often the more important. Vinternberger exposed one of the blastomeres of the frog embryo in the two-cell stage, protecting the other by a lead screen. The embryo developed normally until gastrulation, when the irradiated half ceased growth while the other half continued. Packard has shown that irradiated *Drosophila* larvae may continue to grow until the time of pupation and then first show the effects of irradiation. Species and individuals of the same species vary in their sensitivity to irradiation. *Nereis* eggs are more resistant than sea-urchin eggs. The same organism is not equally resistant at different periods in its life; thus, Manor has shown that the eggs of *Drosophila* are killed by small doses of X rays, the larvae require more, the pupae still more, and the adults succumb only after very large doses. Different tissues of the same organism are unequally affected by both X rays and radium emanations. L. Loeb states that generative organs (ovaries and testicles) are most sensitive to irradiation. Lymphocytes are also destroyed by very small doses of X rays.

Disturbing the winter's rest of plants has long been of interest to the botanist. Must the plant rest a definite or minimum length of time before it can blossom forth again? Is the "sleep" a very sound one, or can it be disturbed easily? These questions Weber has attempted to answer through the use of X rays. His work had its origin in some experiments by Hans Molisch, which showed that radium emanations would end the resting period of winter buds and bring them into leaf. As the alpha rays were screened out by glass, the beta or gamma rays were probably responsible. Weber continued the work, using X rays, and found that they caused resting buds of *Syringa* to open prematurely. A too heavy dose caused the growing buds later to fall.

Some interesting work has been done upon the effects of X rays on the hereditary characters of plants and animals. Among the first outstanding pieces of research of this sort was that of Goodspeed, working on tobacco, and that of Muller, with the fruit fly, *Drosophila*, as his material. The former found that when the reproductive cells of *Nicotiana*, whose many (24) pairs of chromosomes might well exhibit irregular behavior though actually very few mutations occur, are subjected to X rays and radium emanations, chromosome disturbances are rather frequent and result in changes in leaf and flower form and plant size.

Muller, in his work on the fruit fly, found that mutations, or permanent changes, particularly in the eyes, can be induced by radiation with X rays. The changes cannot be distinguished from those arising spontaneously.

Other portions of the spectrum having wave lengths shorter than X rays produce little effect upon the genes, or hereditary units of organisms, but longer rays, *viz.*, the ultraviolet, have been shown to have an effect similar to that of X rays.

Alpha, Beta, and Gamma Rays.—Of the three types of radiation from radium—alpha and beta particles and gamma rays—only the last is included in the spectrum of electromagnetic radiation. It is customary to refer to alpha and beta particles as particles, but as beta radiations are free electrons, the distinction between particle and wave is not definite. As a result, beta radiations are often referred to as rays, though neither they nor alpha particles have yet been given a place in the spectrum.

Alpha particles are of no special medical value but may influence vital processes. R. E. Zirkle has shown that alpha particles emitted from polonium (radium F), at an average rate of 100,000 particles per second, retard or completely inhibit the germination of fern spores. The effect is more severe when the nucleus and not the cytoplasm alone is irradiated.

Beta and gamma rays also ordinarily affect plants in such a way as to retard growth. Blaauw and van Heyningen find this to be true of certain fungi.

The usually observed effect of radium radiation on living matter is a harmful one, but certain forms are extremely resistant; this is true of Protozoa and even more so of Myxomycetes (slime

molds). The plasmodia of Myxomycetes radiated in culture with radium needles (five 12-mg. needles in a 9-cm. dish) show no harmful effects whatever but grow luxuriantly—often better than the controls. While usually avoiding direct or constant contact with the needles, the protoplasm may actually entwine them for an hour or more at a time (Fig. 173). No suppressed ill effect is to be observed in the future cultures of the radiated material, nor does the protoplasm itself, under microscopic examination, show any change. This is true only in culture where the protoplasm of the mold can avoid close proximity to the radium, but damage results when a small piece of plasmodium is kept within 1 mm. of the radium (nine needles totaling 108 mg.)

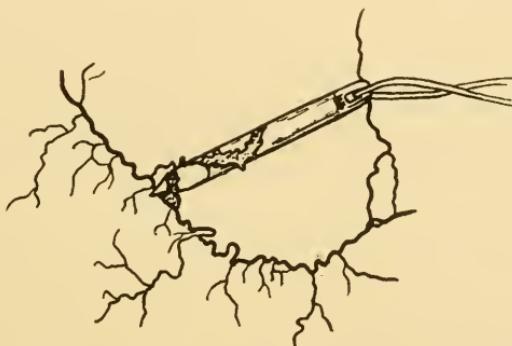


FIG. 173.—The protoplasm of a slime mold entwining a 12-mg. radium needle. for long periods of time; however, the protoplasm continues streaming for 3 hr. during treatment and may still be alive and capable of renewed growth when removed to a culture medium after 20 hr. of treatment.

Animal cells usually react unfavorably to radium radiation, but the extent of the reaction is dependent upon other factors. Both Packard, on *Drosophila* eggs, and Strangeways and Fell, on chick tissue cultures, found that if two lots of cells are irradiated, one at a high and one at a low temperature, the death rate is much greater in the former (owing to greater metabolic activity).

The use of radium rivals that of X rays in medical therapy. Of the three types of radiation, gamma rays are the most effective, though beta particles are sometimes used. Gamma rays serve the surgeon in the treatment of cancer. Beneficial results have undoubtedly been obtained from the use of radium, but the benefit has not been so great or so permanent as had been hoped.

Radium does kill tissue and retard the growth of cells, and young (cancer) cells are more sensitive than old ones, but one cannot be certain that all cancer cells receive the emanations. In any case, the cancerous growth is retarded, and life usually prolonged somewhat by treatment.

Hereditary changes similar to those caused in plants and animals by radiation with X rays have also been produced in part by radiation from radium. Especially interesting is the work of Babcock and Collins, who allowed fruit flies to breed in a locality where there was fully twice the natural earth radiation that existed in the laboratory. Such flies develop a tendency that causes the males before hatching to die twice as often as do normal flies. From this we may, with some justification, conclude that radioactive mineral deposits lying near the surface of the earth affect the course of evolution.

Cosmic Rays.—Lastly come the cosmic rays, too new in our knowledge of radiation yet to have been put to use. But predictions as to their influence on life have already been made. John Joly suggests that as young and cancerous tissue appears to be more sensitive to gamma, and therefore probably also to cosmic, rays than is normal adult tissue, it is possible that our bodies are kept relatively free from cancer by the bombardment of the rays which they are constantly receiving. Should this feature of our radiation environment be lessened, as it may be during certain (rhythmic) periods, then such times would be ones of increased cancer growth. The speculation is highly imaginative, but who can deny the possibility of some truth in it? Twelve cosmic rays a second strike our bodies (at sea level).

We thus see that experimental evidence is overwhelming in support of the hypothesis that the evolution, development, and well-being of plants and animals are associated with and in great measure dependent upon their natural radiation environment. To this can be added the internal environment of tissues, with its content of radioactive elements such as potassium and rubidium.

LIVING MATTER AS A SOURCE OF RADIATION

So far, we have considered the influence of our radiation environment upon life. Let us now consider living matter as a source of radiation.

Heat is the most familiar form of radiation from organisms. It is so familiar that we take it for granted.

Luminescence among insects and fish is not uncommon—the firefly is known to all. The sources of this light are two. Luminous bacteria may be responsible. This is true of certain tropical fish the light organs of which are colonies of luminous bacteria. The fish thus has nothing to do with its light, which goes on shining interminably. Usually, luminescence in organisms is due to a reaction between the two chemicals which duBois called *luciferin* and *luciferase*. When these come into contact, light results. E. N. Harvey has extended this work.

Fluorescence in physical systems was first interpreted by Stokes in 1852. Helmholtz soon after made a study of the fluorescence of the lens of the eye and considered the wave lengths, 3,000 to 4,000 A. U. to be the most effective in producing the effect. Fluorescence is the transformation of light from that form which illuminates matter into that radiated from it, so that the radiated light is of a different (greater) wave length. Fluorescence in organisms has received considerable attention of late from F. E. Lloyd, who has found that the blue-green algae, the diatoms, and some of the green algae (the Pleurococcaceae) are strongly fluorescent when viewed ultramicroscopically with a dark-field condenser, if the proper optical conditions are achieved. The fluorescence is due to phycocyanin rather than to chlorophyll. Lloyd has also reported fluorescence in a number of other unicellular organisms. He found *Paramoecium*, *Oxytricha*, and certain bacteria to fluoresce in monochromatic light.

Phosphorescence is another form of radiation from living things. Phosphorescence, where due to exposure to heat, light, or electricity, differs from fluorescence in that it continues after illumination has ceased. Where due to phosphorus, it is the result of oxidation. It is in this form that it is best known in organisms. It is to be observed particularly in minute marine organisms which at night may make the oar of a rowboat resemble a flaming torch in the water.

Electricity is a form of energy released by living matter to which reference has already been made (page 355).

Gurwitsch Rays.—The foregoing types of radiation are rather familiar and have long been known. They are, therefore, not questioned, and as something of their physical and chemical

nature is known, they have long since ceased to be enshrouded in mystery. The production of light and electricity by animals might well be questioned by the layman, if not by the scientist, were not both phenomena of common experience. We turn now to a form of radiation from organisms which is not conspicuous and has but recently been discovered.

In 1923, the Russian histologist Alexander Gurwitsch discovered that living cells give off a type of radiant energy which may stimulate other tissues to more active growth. The existence of such energy has been questioned, although it is supported by experiments of considerable diversity, carried out in a number of laboratories.

The original experiment of Gurwitsch was done with the roots of onions. One root was held in a horizontal position close to and pointing directly toward another held in a vertical position. After some hours, the tip of the vertical root, the so-called receptor, was "fixed" (killed with chemicals), sectioned, and subjected to microscopic examination. It was found that in the receptor root there were more cells in the process of division on that side which had faced the sender than on the opposite side, indicating that the sender root had radiated some form of energy which accelerated cell division, or growth, in the receptor.

Gurwitsch rays are not limited to the onion; they appear to be characteristic of all living matter under certain (metabolically active) conditions. That this is true is indicated by the long and varied list of tissues so far known to radiate. The following cells, tissues, and organisms have been shown to be sources of radiant energy: bacteria, yeast, *Hydra*, the eggs of lower animals, plant seedlings, potatoes, beets, blood of man, frog, and rat, cancerous tissue, muscle, nerve, the brain of young axolotls, a mash of *Drosophila* larvae, a mash of tadpoles, and regeneration processes after amputation in tadpoles. Malignant tumors emit rays in the strongest fashion; benign ones, less.

A very important feature of the radiation, which answers a question that must inevitably arise in connection with the transmission of the energy through tissue, is that of secondary radiation. It is the key to the whole problem. Without it there can be no induction effect, as surrounding tissue would rapidly absorb the radiation from within. Lacking secondary radiation, the primary form would never reach the outside, except where

it is superficial in origin. Also, a cell may not in itself be capable of radiation but may be aroused to emanation activity by a neighboring radioactive cell. The secondary radiation may be greater than the primary.

The number of known kinds of receptors has not, strange to say, increased with that of the senders. Yeast is the most satisfactory receptor. Bacteria also respond well.

The work of Gurwitsch has been so severely criticized that it will be well to let the matter rest until future research establishes its truth or falsity. Numerous investigators in Russia, Germany, Holland, and elsewhere support the work with experiments of their own, but others are unable to prove the results.

In judging the value of criticism, we must distinguish between a purely emotional reaction without experimental work to support it and carefully carried out research. Most of the opposition to Gurwitsch is of the former sort, though some is of the latter kind. The problem is too interesting a one to let it go without some, if need be purely theoretical, consideration.

The hypothesis that living matter gives off radiant energy may be approached deductively. The chemical constituents of protoplasm include potassium and rubidium, both slightly radioactive. Knowledge of the presence of these elements in living matter is of long standing. Traces of radium have recently been found in living plants and animals. McCollum showed that potassium is mobilized where mitosis is at a maximum (this was before it was known that potassium is radioactive), and Kroetz showed experimentally that X rays and ultraviolet are effective in mobilizing potassium at the expense of calcium. If we assume the potassium content of muscle tissue to be 0.3 per cent, we can account for beta radiation alone equivalent to a possible maximum of 36,000 light quanta per square centimeter per second. Most of this radiation probably appears as secondary energy of a different wave length.

Every chemical reaction involves a transfer of energy which may manifest itself in the form of heat, light, or an electrical potential. The chemist is quite familiar with radiations that produce fluorescence, *e.g.*, sodium chloride crystals when exposed to roentgen rays. Fluorescence also occurs when sodium is burned in the presence of chlorine. Chemoluminescence is an ever growing chapter in present-day chemistry. W. C. Lewis

and Perrin have suggested that all chemical reactions are photochemical in character. They assume that a molecule does not react until it becomes activated by radiant energy. This assumption—that every simple chemical reaction is accomplished by the adsorption of light of one frequency—involves also the assumption that light of another frequency is emitted. The hypothesis enabled Perrin to give an explanation of the phenomena of photo- and thermoluminescence. While it is unlikely, as G. N. Lewis says, that all chemical reactions are due to the influence of light and not in part to molecular activity (thermal motion), it seems very likely that the emission of light is a frequent characteristic of chemical reactions. Solidified nitrogen emits an intense luminescence when exposed to electric rays; thus does a single chemical element have the properties of a phosphorescent body.

F. Daniels, in a discussion of photons in chemistry and biology, states that, as the phenomenon of chemical luminescence is of not infrequent occurrence in nature (in decaying wood, bacteria in sea water oxidized as a boat plows through the water, etc.), there is no fundamental reason why some reactions in protoplasm should not emit photons. Any chemical reaction that gives rise to the displacement of an electron (or atom) in a molecule will cause a photon of radiation to be emitted when the electron falls back into its position of lower energy.

The emanation of radiant energy from living matter in the form of ultraviolet light (which is said to be the nature of Gurwitseh rays) is no more remarkable than the giving off of heat, light, and electricity by organisms. Because these latter are of such common experience, we unhesitatingly admit their existence. It is but a step from heat, light, and electricity to such other forms of radiant energy as ultraviolet rays, roentgen rays, radium radiations, and cosmic rays. While certain of these manifestations of energy are less well understood than others, and in this sense are more mysterious, yet none is vitalistic except in so far as it is given off by living matter. From every point of view, each of them is as physical—as materialistic—as are the others. The presence of radiant energy in protoplasm is one of the least mysterious things about it. We need think only of movement, growth, and reproduction to realize this.

The work of Gurwitsch and his collaborators may prove to be erroneous. Misleading factors may be involved of which all the experimenters are ignorant. If it should be necessary later to make substantial modifications of the discovery of Gurwitsch, we shall yet be forced to grant that the giving off of a form of radiant energy by protoplasm is not merely a possible but a very probable event.

CHAPTER XXI

THE ROLE OF WATER

Water, as the solvent or dispersion medium of all other constituents of protoplasm, becomes one of the most significant of the components of living matter. It is the solvent, the carrier of the reacting substances. It serves not only as the medium in which reactions take place but as the vehicle by means of which needed substances are brought to the cell and waste products carried away.

The role of water in living processes is so great that a meager chapter such as the present one cannot possibly do it justice. The ascent of sap in trees, the part that water plays in that most fundamental of all organic reactions photosynthesis, its part in the metabolism of the human body are all problems that cannot be adequately dealt with here. Brief mention of some general problems in water metabolism, with special reference to the role of colloidal water, is the limited scope of this chapter. The high surface tension of water, its tendency to form hydrates, its high dielectric constant, its high specific and latent heats, but, above all, its great power as a solvent make it the *sine qua non* of biological processes. Water lubricates and protects the organs of the body; the tendon sheaths are protected by water; the synovial and cerebrospinal fluids are lubricants. Absorption, secretion, diffusion, and excretion could not take place without water. Water exists not only as a pure solvent but as water of hydration, combined with carbohydrate and protein. In addition to water taken into the body, there is water formed by the polymerization or synthesis (the reverse of hydrolysis) of compounds and by the oxidation of hydrogen-containing substances. Such water is known as *metabolic water*. In the case of the combustion of a fat, slightly more than its own weight in water is produced. From 10 to 14 grams of metabolic water are formed per 100 cal. in the ordinary diet.

The amount of water in organisms is great (about 75 per cent), but greater still is the amount being constantly lost and added.

A plant is said to require 200 to 400 lb. of water to produce a pound of dry matter; and the circulation of water in animals is enormous. So great are the incoming and outgoing of water in organisms that the amount in tissues would appear not to be precisely maintained. But this is not the case. On the contrary, the balance between water and the other constituents is very accurately controlled. Water is not free to enter a cell to an unlimited extent. We ordinarily think of it as passing freely into and out of the cell—because we are in the habit of regarding the plasma membrane as wholly permeable to water—but this is not true. There is, as Höfler points out, a very accurate control. This regulatory power of protoplasm is assumed to be parallel to that of jellies, *e.g.*, gelatin, which swells a definite amount in water and no more (see hysteresis and miscibility, pages 145, 207). Such a viewpoint ascribes the entrance of water into protoplasm to imbibition forces. Water enters plant cells principally by osmosis, in the pure and strict sense; osmosis is thought by some to be also primarily responsible in the case of animal cells (see page 196).

Just as the water supply of the cell is very accurately controlled by the regulatory power of the membrane or the protoplasm as such, so also is the quantity of water taken into the body controlled, an excess or a deficit being harmful. A fasting animal may lose all its fat and half its protein but not more than one-tenth of its water.

Where a disturbed metabolic condition results from too little or too much water, the changed water content may be only the first cause leading to a second disturbance which is the final cause. This is true in the case of miner's cramp (page 450), where excessive loss of water (sweating) is the primary cause, leading to decreased salt content, the ultimate cause of the cramp.

Of the many evident uses of water in the animal body—as the medium for metabolic processes, the distribution of foods, the regulation of temperature (through circulation and evaporation from the skin), keeping mucous surfaces moist, eliminating poisons from the kidneys, etc. only a few instances can be selected for consideration.

McQuarrie, Trumper, Fischer, Beutner, and others call attention to the importance of water metabolism in connection with epilepsy, diabetes, edema, and kidney function.

One of the most common examples of a disturbance in water control in animals is the condition known as *edema*, or swelling of tissues. Knowledge of the forces involved in the movement of water through capillaries and membranes and the adsorption of water on to surfaces has advanced sufficiently to make specific statements possible on so complicated a medical problem as the cause of edema. Ordinarily, edema will develop when the normal permeability of the capillary wall is altered or when there is a disturbance in equilibrium between the hydrostatic pressure and the osmotic pressure of the blood. A common cause of altered permeability is inflammation. Chemical or mechanical injury of the capillary blood vessels may also cause edema. Normally, the hydrostatic pressure of the blood balances the osmotic effect; but when this balance of forces is disturbed in either direction, edema develops. Thus, an increased hydrostatic pressure gives rise to a loss of fluid from the circulating blood to the tissue. This is known as cardiac edema. In so-called nephrotic or hydremic edema, due to the increased permeability of the capillaries of the kidneys, there is a leakage of blood proteins. This persistent loss of plasma protein accounts for the decrease in the osmotic effect. As long as the plasma protein content is above 5 per cent, with a specific gravity of 1.023, there is little likelihood of edema. Below this critical level, there is a definite tendency toward edema. Deficit in plasma protein and its associated edema may also be caused by a diet low in protein. Edema is present in various diseases, whenever there is starvation, malnutrition, or excessive catabolism, as in toxic conditions with high temperatures. In postoperative or generalized edema, the problem is more complicated and as yet not fully understood. The primary causes of edema may be numerous and quite different from one another. It is possible that the ultimate cause in many cases is a change in acidity.

Epilepsy is associated with an excessive water supply and alkalinity, while diabetes is associated with dehydration of the tissues and acidity. Surplus water in epilepsy collects in the subarachnoid spaces. To bring the water balance down to normal is the task of the physician. Epileptic convulsions usually stop when a negative balance in body water has been obtained, while a positive water balance will precipitate epileptic seizures. Convulsions also cease after partial fasting or a

ketogenic, *i.e.*, high fat, low-carbohydrate, diet. If there is less than one molecule of glucose for every two molecules of the fatty acids in the diet, beta oxidation of the higher fatty acids is incomplete with excessive formation of ketone bodies. Any factor that reduces the semipermeability of the brain cells, *e.g.*, dehydration, acidosis and ketosis (increase in ketone bodies, such as acetone, acetoacetic acid, and beta-hydroxybutyric acid), retards the accumulation of fluid in the brain cells and the subarachnoid spaces and thereby reduces, if it does not prevent, the convulsions of epilepsy. During starvation, the largest loss in body substance is water, with the production of a ketosis, because of the small reserves of carbohydrates in the body. There is uncertainty as to whether water loss or the disturbance of the acid-base equilibrium is the primary factor in convulsions. But we do know that a ketogenic diet decreases the permeability of cell membranes and that a definite depletion in extracellular fluid results.

Diabetes is due primarily to faulty sugar metabolism, but it is also constantly associated with those conditions that alleviate epileptic convulsions such as ketosis, loss of water, and alkalinity. The diabetic is exactly the reverse of the epileptic; he is dehydrated, while the epileptic is hydremic. Theoretically, a person suffering from both epilepsy and diabetes should be extremely rare; such is the case.

Aging may in large measure be due to the inability of protoplasm to hold water, for as we grow old there is a decrease in water content of the body. Attention has been called to this under syneresis (page 146).

Gortner has given special attention to the role of water in plants, from the point of view of the state in which water exists in the plant body, *i.e.*, whether free or bound. By free water is meant water in bulk. Bound water is water held to organic substances by adsorptive or other forces. Only free water is available for functions such as the translocation of food, the photosynthesis of sugars, transpiration, sweating, the elimination of toxic and waste products, etc. The chemical state of bound water in the organism is not known. Even so old and familiar a state as water of hydration is not understood fully. Bragg believes that water of crystallization is so intimately bound to the crystal molecule that it no longer exists as water but that,

instead, a new molecule results (page 176). However intimate or loose the association may be in hydration, we can at least assume that the water molecule is actually held, and more firmly in some cases than in others. Water of hydration may often be driven off by a mild application of heat, while at other times it is separated only with great difficulty. Water held osmotically, in the strict sense, is free water (though obviously it is not free to move against an osmotic gradient). Water held by imbibition forces is usually bound water. To squeeze the water out of some gels requires terrific forces, and not always then can it be done. No known force will keep gelatin or starch from taking up water.

Bound water may also be defined as water that will not freeze; this serves as a method for determining the amount of bound water present. The problem of a bound \rightleftharpoons free water equilibrium in biological systems has centered primarily around the question of freezing—the so-called frost resistance or winter hardiness of plants and animals. When reduced to its simplest form, it is a problem of the cold resistance of protoplasm. The survival of a cell at low temperature appears quite definitely to rest upon the relative amount of free and bound water. Bound water does not freeze; free water freezes readily, forms large ice crystals, and these, simply because of size and mechanical pressure, kill the cell. Death is, therefore, not due to a freezing of the protoplasm itself, *i.e.*, freezing of the water held by adsorption to organic matter in the cell. In their studies on the proportion of bound and free water in gelatin and egg white, Jones and Gortner found that all the water that would freeze was frozen when the temperature reached -6°C . and that no additional water froze even when the temperature reached -50°C .

The problem of bound *vs.* free water has a practical application in the study of winter hardiness in plants. Free water freezes readily. Bound (adsorbed) water does not freeze. The relative proportion of free and bound water determines drought and frost resistance (winter hardiness) of plants and animals. Increase in the concentration of dissolved substances (sugar), which lowers the freezing point of aqueous solutions, would be one effective way for plants to survive great cold, but still better is it to bind the water to proteins and other colloidal substances by adsorption.

The tenacity with which tissues hold imbibed (adsorbed) water is illustrated by an example of R. Newton. The succulent stems of a cactus were put in a desiccator (drying chamber) over sulphuric acid. After six months, in an atmosphere of almost zero humidity, the juicy stems had lost but 10 per cent of their water. The stems remained dormant and, on being returned to a moist atmosphere, sprouted. Continuing his studies on this problem with Gortner, Newton found a direct correlation between winter hardiness (resistance to freezing) in wheat and percentage of bound water, the explanation being that as adsorbed water does not freeze, while free water does, the more of its total water supply a plant can hold in the bound state the more able it is to withstand low winter temperatures. W. Robinson carried this field of investigation over into the life of insects and found there the same correlation. The pupae of two hardy species of insects which, in cocoons, are exposed to low winter temperatures, gained in bound water and lost in free water under the stress of falling temperature, while a nonhardy weevil which cannot survive below freezing lost bound water in a falling temperature. Robinson's conclusion for insects is the same as that of Newton and Gortner for plants, *viz.*, that since bound water will not freeze at low temperatures, the greater the quantity of water that can be converted into that form and the more rapidly it can be done the greater is the protection against freezing afforded to that species.

The foregoing hypotheses are undoubtedly true in part, but resistance to drought and freezing in plants and animals is probably more than a question of relative amounts of free and adsorbed water; other factors enter in, such as the waxy coverings on cacti, which are a great protection against the loss of water. Furthermore, some plants and fruits with much free water, such as tomatoes, resist freezing at fairly low temperatures.

Newer concepts concerning the hydrogen ion lead to the belief that when water reacts with an acid, $\text{H}_3\text{O}^+ + \text{Cl}^-$ are formed (page 301). It has been suggested that so-called bound water may be H_3O in distinction to H_2O .

In spite of questions and criticisms, the concept of bound water and its biological significance is becoming more and more firmly established.

Heavy Water.—The discovery of an isotope of hydrogen led immediately to its application to biology.

Isotopes were first described by J. J. Thomson, who called attention to the fact that neon may exist in two forms with properties alike in all respects, except weight. His discovery was followed by the work of Aston to whom most of our present knowledge of isotopes is due. Lead that has come into existence through the breaking down of uranium has an atomic weight different from that of ordinary lead and also from that of lead which is the result of the breaking down of thorium. These three substances are all lead in that they have the same chemical properties but are of different atomic weights, by which is meant that their atomic nuclei are of different composition but their outer fields of electrons are the same. Such substances are isotopes, and it is now known that the majority of the elements consist of isotopes. The atomic weights of the isotopes of an element are whole numbers; a mixture of them usually results in a non-integral value; thus, chlorine, as we know it, has an atomic weight of 35.5 because it consists of an isotope of weight 35 and one of weight 37. Magnesium has an atomic weight of 24.32, which is the net average of three magnesium isotopes having the respective weights 24, 25, and 26. Among the 92 elements, 66 have been studied as to their possible isotopic character and only 24 have been found to be "simple" elements. Mercury is an extreme case, with six isotopes of atomic weights—197, 198, 199, 200, 202, 204—the net average of the mixture being 200.6, the atomic weight of mercury.

The latest additions to isotopes are those of hydrogen, the most significant one of recent discovery having a mass of two. The discovery of the second hydrogen atom, of twice the weight of the former one, was made by Urey and was based on a new line in the hydrogen spectrum. H^2 is the symbol of the heavier of the two hydrogen isotopes. It probably consists of a neutron added to the nucleus of the lighter hydrogen. Ordinary water contains very little heavy hydrogen—about 1 atom to every 6,000 atoms of light hydrogen.

There is yet another isotope of hydrogen (and there may be more) of atomic weight 3, but it is very rare (H^1 is six thousand times as abundant as H^2 , and H^2 is thirty thousand times as abundant as H^3).

With the discovery of an isotope of hydrogen, there came the prediction of G. N. Lewis that "heavy water" would not support life. The two hydrogen isotopes of atomic weights 1 and 2 and the three oxygen isotopes of atomic weights 16, 17, and 18 could theoretically combine so as to form nine kinds of water, all differing in weight. Among the known forms of water are H_2O , $\text{H}^2\text{H}^1\text{O}$, and $\text{H}^2\text{H}^2\text{O}$ (H_2^2O). The freezing point of the last is 3.8° higher and its boiling point 1.42° higher than that of ordinary water. Lewis predicted that this heavy water would not support life and proved it by showing that tobacco seeds do not sprout in it. Others have since demonstrated that lowly forms of animal life do not long survive in heavy water. Trelease, however, finds little effect of dilute heavy water on fungi. Ewart finds an effect, but it is a retarding one on the movement and growth of bacteria and an accelerating one on spore production.

It appears also that plants react differently to water formed by the condensation of steam than they do to water formed by the melting of ice.

CHAPTER XXII

SALTS

Continued life is dependent upon the presence of salts in protoplasm and in the surrounding fluid. Salts serve organisms in their metabolic activities both as constituents of substances formed and as catalysts or activators of reactions. They serve also as physical agents in establishing and maintaining osmotic pressure, concentration equilibriums, and electric balance. No cell or organism will live long in an environment the salt concentration of which differs greatly from that of the natural surroundings. Marine amoebae can be made to grow in fresh water by gradually reducing the salt content of the sea water, and fresh-water amoebae can be grown for a time in distilled water. The small marine fish Fundulus will likewise live for a while in distilled water. But life in such instances is temporary.

The percentage of salts in protoplasm is about 12. The form in which they occur is not known. On analysis, potassium may be found as the ion, but this does not mean that it exists as such in protoplasm. It may occur so, but it may also be bound, *e.g.*, to a protein. Analysis reveals only the presence of potassium.

Until a few years ago, it was taught that there were 10 elements necessary for plant life—carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, calcium, magnesium, and iron—and that some few others, such as iodine, bromine, and silicon, are found in certain plants for the growth of which they may be necessary. But we now know that this number is wholly inadequate.

Sodium is not given in the list of the first 10 elements necessary for plant life. The absence of this element in plant nutrition and its necessary presence in animal food are distinguishing features of the salt needs of these two groups of organisms. Animals, especially herbivorous ones, need common salt, while plants grow well in a solution wholly lacking in it; on the other hand, the element sodium is not infrequently found as a con-

stituent of plants, especially in marine forms. The alga *Valonia* contains in its cell sap one-fifth the concentration of sodium found in the sea water where it lives.

The list of elements occurring in plants and animals, and therefore possibly necessary to life, has lengthened rapidly in recent years. The agricultural chemist McHargue has made many startling additions. He finds that small amounts of manganese, copper, zinc, boron, barium, strontium, lithium, and rubidium are normal constituents of plants grown under natural conditions and that there are traces of nickel and cobalt. Most of these metals are also constituents of animal tissues. Calves' liver contains a surprisingly large quantity of copper (345 parts per million) and a small amount of manganese and zinc. The pancreas presumably contains traces of nickel and cobalt. Among the most recent additions to the list of extraordinary elements in living things is that of radium, discovered in duckweed.

A number of interesting questions arise in regard to the unexpected presence of these metals in living tissues. Why have we been so late in realizing that they are there? How does the organism tolerate those that are highly toxic; and are they all necessary? The first question is readily answered by the statement that the quantities are too small to be readily detected. Living cells deal with concentrations infinitely lower than those with which the chemist is ordinarily concerned. The presence of radium in duckweed was ascertained, but the concentration of this element in the pond water where the plant grows and from which it must get its supply of radium was too low to be detected. The spectroscope, the same instrument used in analyzing the chemical composition of stars, has made possible the detection of very minute quantities of elements. Lundegardh of Stockholm has been actively engaged in such work. Simply put, the method consists in burning the stems, leaves, roots, and seeds of a plant and then passing light through the ash and into a spectroscope; the latter separates the light into its spectrum. Each chemical element has its own particular spectral lines. It is, therefore, possible to measure the amount of the element present by observing the intensity of the lines. The spectrum is recorded on a photographic plate. In the case of infinitesimal amounts of minerals, a photoelectric cell is used as the light-measuring

instrument. Elements can thus be quantitatively determined. Of some elements, as little as 0.0004 gram can be detected.

The quantities of heavy metals present in plants and animals are exceedingly small compared with those of the alkali metals, but they are still sufficient to make one wonder how the organism can tolerate metals as highly toxic as are copper, zinc, and tin. Copper is a striking example of a poisonous element. Its toxicity is so great that in some early experiments by Nägeli the concentration was reduced to an absurdity. Nägeli found that a trace of copper in water (produced by leaving a few copper coins in water for a time) quickly killed the filamentous alga *Spirogyra*. He then diluted the copper water until, by estimation, but one molecule of copper remained in a liter of water, and still the alga died. He rinsed the glass thoroughly and put in fresh distilled water, and the alga still died. The effect was so startling that Nägeli gave to it the special name "oligodynamic." The experiment was a rather crude one, and undoubtedly more copper ions were present than Nägeli thought (they probably clung to the walls of the vessel), but it at least showed that copper is highly toxic in very low concentrations. Other heavy metals, such as silver, are equally poisonous. A silver coin laid upon an agar culture of bacteria will leave a space free from bacteria around it, owing to minute quantities of the metal which have gone into solution. The toxic action of heavy metals is made use of in antiseptics.

Metals probably exhibit their toxicity by a "poisonous" action on the catalytic powers of other metals. Bredig found that 0.000003 gram of colloidal platinum per liter of water produced a marked acceleration in the decomposition of hydrogen peroxide. The slightest trace of a catalytic poison—0.000001 gram of hydrocyanic acid per liter—will impede this action of platinum. Often, however, the reverse situation is true; a trace of a second element is necessary before the benefits of the first are realized, as in the needed presence of copper if iron is to prevent anemia. Poisonous metals are tolerated by plants and animals because the quantity is very minute—in this case, the element may not only cease to be a poison but become a necessity, for it is generally recognized in physiology that substances which in higher concentration are poisonous may be beneficial in very low concentrations—or the element may be in another physical

state from that in which it occurs as a poison—thus, soluble barium, BaCl_2 , is highly toxic; but insoluble barium, BaSO_4 , is not—or the element may be bound to other substances (proteins) in such a way that its harmful influences are suppressed. Whatever the answer to this question may be, it is now known that the heavy elements found in tissues, though poisonous in high concentrations, become not only harmless but in many cases beneficial and necessary in the concentrations in which they occur in plants and animals.

The following list includes those elements so far known to occur in plants and animals (listed approximately in the order of their abundance):

Carbon	Sulphur	Boron	Aluminum	Thallium
Hydrogen	Sodium	Lithium	Rubidium	Titanium
Oxygen	Iron	Barium	Cobalt	Silver
Nitrogen	Chlorine	Strontium	Lead	Vanadium
Potassium	Iodine	Manganese	Nickel	Radium
Calcium	Bromine	Copper	Mercury	Germanium
Phosphorus	Fluorine	Zinc	Arsenic	Gallium
Magnesium	Silicon	Tin	Selenium	

That the first 10 are necessary is graphically shown in the case of plants by the technique known as water culture, with which much has been accomplished by the plant physiologist B. E. Livingston, his students, and others. J. E. McMurtrey has obtained a series of excellent water cultures, showing the rate of growth of tobacco when one of nine elements is omitted (Fig. 174). Carbon, oxygen, and hydrogen are not considered, for they cannot be excluded, as the plant gets them from water and air.

That all of the 37 elements found in plants and animals are absolutely necessary cannot be said, but certainly more than 10 or 12 are needed. Why is it, then, that a plant will do so well in a water culture, such as the control in McMurtrey's experiment (6, Fig. 174), which presumably contains but 10 elements—carbon, hydrogen, and oxygen being otherwise available? The answer may be one of two. Most chemicals contain minute amounts of other elements, in sufficient quantity for the needs of a plant. Only highly refined salts are free of foreign matter. The "trace elements" necessary for plant life may be present as impurities. Or it may be that a plant gets on quite well in the laboratory without this or that element but that when

subjected to the rigors of outdoor life, it succumbs. Thus, laboratory cultures indicate that silica is not necessary for grasses (many if not all of which contain silica) but they develop well without this element only in culture. In the field, silica appears to play the role of a protector from parasites. Wheat and rye, grown in laboratory cultures deficient in silicic acid, suffer severely from "rust."



FIG. 174.—Tobacco plants in culture solutions: 1 without nitrogen; 2 without phosphorus; 3 without potassium; 4 without calcium; 5 without magnesium; 6 all of the necessary elements included; 7 without boron; 8 without sulphur; 9 without manganese; 10 without iron. (From J. E. McMurtrey.)

We now come to the function of these elements. The first four in the preceding list, *viz.*, carbon, hydrogen, oxygen, and nitrogen, are necessary for the manufacture of foods, such as sugars, fats, and proteins.

Potassium.—That potassium is necessary for plant life is apparent from the fact that it has long been recognized as one of the three principal fertilizer constituents, nitrogen and phosphorus being the other two. The role of potassium in organisms appears to be primarily a catalytic one, as is true of numerous other elements that do not enter into the composition of tissue or serve directly as food for producing energy.

Calcium.—The role of calcium is important though not always definitely known. It serves, with phosphorus, in bone production. The calcium-phosphorus balance is of prime significance to the growing child (see page 515). Calcium seems to have other influences on the animal body, for when the supply is deficient man becomes irritable. Its supply to the blood appears to be controlled by the parathyroid glands.

Blood serum in health contains about 10 mg. of calcium per 100 cc. of serum, of which about one-fifth exists as free ions, the remainder being bound. Nearly half of the total calcium is presumed to be bound to proteins and lipins. The calcium content of the blood is kept very constant, so that the body mechanism must be an accurately controlled one in order to maintain so perfect a balance between the absorption, use, and elimination of calcium. The normal calcium requirements of a healthy adult (0.5 gram per day) are increased during growth, pregnancy, and lactation.

The role of calcium in plant life has long been assumed to be a catalytic one, but it may, so Hansteen-Cranner believes, be utilized directly in the formation of the cell wall (of root hairs). It is apparently the only constituent of the cell wall not synthesized in the plant. R. True also assumes that calcium is needed for cell-wall formation, possibly as a constituent of the wall in the form of calcium peetate or as a catalyst in cellulose synthesis.

Phosphorus.—The part played by phosphorus in the living world is great. Its prime role in plant life is as phosphates in the soil, where the plant obtains these salts for its nutritional requirements. Phosphorus occurs in organisms as phosphates, phosphoric acid, and phosphoproteins. Tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, is the principal mineral salt of bone. Phosphorus is often bound to sugars; as hexose phosphate it occurs in the blood, where it is also the chief ingredient of the enzyme phosphatase. The role of phosphorus in muscle metabolism is of recent discovery (page 458).

Magnesium.—The function of magnesium, long known to be a necessary element in metabolic processes, is only just coming to light chiefly through the experiments of McCollum, which indicate that a diet lacking in this element causes convulsions and death in the majority of cases (in rats).

Sulphur.—Sulphur is a constituent of, and therefore necessary to, the synthesis of proteins in plants. It has occupied a prominent place in the economy and thoughts of man for ages. We find it referred to in the relation of fire and *brimstone*. Among the alchemists, sulphur played an important role in their endeavors to transmute base metals into silver and gold. In agriculture, sulphur, as such or in simple compounds, has long been found of value in aiding the growth of vegetation and in checking parasites. In medicine, it has been found of use in skin applications. Within recent years, certain sulphur-containing organic compounds, in particular cystine and its reduced form cysteine, have become recognized as necessary ingredients in diet. A diet lacking in cystine will not support life. (The hair, the skin, and the nails are in large part made up of cystine in complex form.)

Quite recently, it has been found that the cysteine complex glutathione plays a prominent role in cellular metabolism. Insulin, the pancreatic hormone regulating sugar metabolism, is such a complex. Other sulphur-containing compounds have come into prominence of late. The active principles of the posterior pituitary gland (page 509) contain sulphur as a vital part of their chemical constitution. It becomes more and more evident that sulphur, free and combined, is exceedingly important in health and disease.

The significance of another sulphur combination, *viz.*, the sulphhydryl radical, SH_2 , will be considered elsewhere (page 520).

The sulphur bacteria are an outstanding instance in which nature has raised the element to a high position in the physiology of the cell. These bacteria use sulphur instead of carbon in the synthesis of higher foods.

Sodium.—Sodium undoubtedly plays a more important role than that usually ascribed to it, *viz.*, as a physical agent in maintaining equilibrium (osmotic, electric, etc.), but we are unaware of its full role. Sodium chloride was early known to be a constituent of all animal fluids and was experimentally used as a medium in which to bathe organs and tissues for study in the living condition.

It has long been observed that in patients suffering with Addison's disease, in which the adrenal glands are not supplying an adequate amount of secretion for the body's needs, the amount of sodium chloride in the blood is considerably below normal. The

patient's condition is improved when extra common salt is taken with the food.

Iron.—Before the recent work on sulphur raised it to pre-eminence as a catalyst in vital reactions, iron held this position. Iron is necessary in plants for the production of chlorophyll. Without it, leaves become chlorotic, *i.e.*, suffer from chlorosis, or lack of green color. While it is true that iron deficiency is a cause of chlorosis, there is usually ample iron in the soil to satisfy the modest needs of the plant. The chlorotic condition is most often due to other factors (alkalinity) which make it impossible for the plant to use the available iron (see page 322).

Iron is present in animals as a constituent of blood, where it presumably serves as a carrier of oxygen. Otto Warburg regards respiration as a cycle in which molecular oxygen reacts with bivalent iron, whereby iron in a higher state of oxidation is formed. Respiration, pure and simple, takes place within cells, but in its entirety it involves oxygenation within the lungs, where molecular oxygen unites with hemoglobin without atomic cleavage; there is no transfer of electrons or hydrogen atoms. The reaction is consequently not comparable to ordinary oxidation. (In distinction to oxyhemoglobin, to which we have just had reference, methemoglobin is a true oxidation product of hemoglobin. Methemoglobin cannot take up molecular oxygen and serves no purpose in respiration.) Acting thus simply as a carrier of molecular oxygen, hemoglobin enters the tissues of the body, and here the oxygen that it carries reacts with the divalent iron of a respiratory enzyme (a ferment, pigment, or oxidase), converting the Fe^{++} into the trivalent Fe^{+++} .

The amount of iron in different types of cells has been determined and found to be 0.1 to 0.01 mg. per gram of cell substance. Warburg considers the possibility of other heavy metals functioning as the respiratory catalyst. Copper and manganese would apparently serve equally well. These elements occur in protoplasm, but their amounts are too minute to account for the rate of oxygen consumption in respiration (for example, there is 1 gram of cell substance to 0.0001 mg. of manganese—a hundred to a thousand times smaller than the iron content.)

Iron has long been known to be beneficial in cases of anemia. Now it is evident that copper must also be present, presumably as a catalyst.

Chlorine.—Chlorine is an element, usually present and often abundant, that does not appear to be necessary for all kinds of life, though as hydrochloric acid it is an important constituent of the gastric juice. Its excessive presence may be objectionable. Potatoes grown in soil rich in chlorine contain less starch than those grown in soil deficient in this element. Chlorine fertilizers are, therefore, to be avoided.

Iodine, Bromine, Fluorine.—The presence of iodine, bromine, and fluorine in algae (the brown algae are the commercial source of iodine) leads one to assume that these elements are necessary to certain plants; of course, the case may as well be one of tolerance as one of need; the latter, however, seems the more probable. In animals, the need of iodine is well recognized. It has of late played a prominent part in the prevention of goiter.

Silicon.—Silicon is present in plants, *e.g.*, in grasses and the scouring rush, *Equisetum*, but rarely has a prominent physiological role been ascribed to it. However, there is apparently a need for silica in plants as a protection against disease.

Boron.—Boron is a rather unexpected element to be raised to the importance in plant life that it has of late attained through the work of Winifred Brenchley in England and of McHargue in America. Both prove boron to be necessary in the nutrition of plants and incapable of being replaced by any other element.

The work of T. Schmucker on boron is interesting. He attempted to germinate the pollen of the water lily in artificial media as nearly as possible like the liquid occurring on the top of the pistil where the pollen grains grow. Many attempts failed, but the ash of the liquid, taken from the pistil and added to 1 per cent glucose, was as effective as the original liquid. The active element proved to be boron. Boric acid, H_3BO_3 , occurs in the pistil fluid. In artificial media, 0.01 mg. of boric acid in 1 cc. gives good results; even 0.0005 mg. acts favorably. Schmucker regards boron as an inorganic hormone, or catalyst.

Lithium.—Lithium is widely distributed in plants but in greatly differing quantities. Haeddon finds that alfalfa stores little lithium (and much strontium), while tobacco stores much lithium (and little strontium). Nakamura finds lithium salts to exert a stimulating action on plants (barley and peas).

Barium and Strontium.—Barium and strontium are interesting because of the controversy that has centered around the question,

Is one element replaceable by another in the nutrition of a plant? We shall later see that it is not. Were it so, then barium and strontium, both closely related to calcium, should replace the latter in plant nutrition. Both are far more toxic than calcium, particularly barium; it is therefore obvious that they cannot replace calcium. They do, however, occur in plants and animals, though their function is unknown.

Manganese.—McHargue has found that when plants are grown in purified sand cultures from which manganese has been carefully excluded and in which pure compounds of the so-called 10 essential elements are present in available form, the plants make no further growth after the food material stored in the seeds has been exhausted. The addition of a small amount of manganese produces normal growth.

As for animals, McCollum makes it quite clear that manganese is necessary. Rats restricted to a diet free from manganese grow well, but while they produce normal litters, they do not care for their young, and the latter die from neglect.

Copper.—Copper is another metal that has come to the front as a necessary constituent of diet. Its chief value to human health is its effect on the availability of iron as a cure for anemia. In fact, copper dissolved in milk is alone effective in the regeneration of hemoglobin, though more so when accompanied by iron. In plants, copper is necessary (or at least beneficial) to chlorophyll formation. Thus do both iron and copper stimulate the production of the chief pigments of plants and animals, which brings further evidence to bear on the analogy between chlorophyll and hemoglobin. Other experiments, such as those of Nägeli, emphasize the high toxic effect of copper. Small aquatic plants are not harmed by concentrations of 1:100,000,000 of copper but suffer a pronounced reduction in growth by a concentration of 1:50,000,000.

Zinc.—A. L. Sommer has found zinc necessary for the continued life of barley, wheat, etc. Zinc appears also to be beneficial to microorganisms living in the soil. It may be necessary for higher animals, as it occurs in calves' liver.

Tin.—Tin is said to occur in some body organs. Its use is not known.

Aluminum.—Uncertainty has arisen over the presence and function of aluminum in plant and animal matter. The quantity

is very small, but the claim is made that its presence has been definitely determined in potato, lima bean, peach pit, egg, beef, and human cancerous tissue. The blue color of the flowers of Hydrangea when grown on certain forest and moor soils may be due to the presence of aluminum salts; these may also be artificially added to the soil (though hydrogen may be the responsible element here). The metal has an effect, but it cannot be said to be either beneficial or harmful.

In General.—R. W. Thatcher has classified into eight groups the elements found in plants. He adds that all the elements known to have any function in plant nutrition occur in the first four orbits of the periodic table. The groups are (I) hydrogen and oxygen, energy-exchange elements; (II) carbon, nitrogen, sulphur, and phosphorus, energy storers; (III) sodium, potassium, calcium, and magnesium, translocation regulators; (IV) manganese, iron (cobalt, nickel), copper, and zinc, oxidation-reduction regulators; (V) boron, aluminum, silicon, arsenic, selenium, functions unknown; (VI) chlorine, fluorine, bromine, and iodine, functions unknown; (VII) cobalt and nickel, functions unknown; (VIII) germanium, gallium, and other rare elements, functions unknown.

The Salt Environment.—Claude Bernard reminded us that the living cell could not be considered apart from its environment. The chief constituent of this environment is water, and the second in importance is its salt content. As far back as 1773, Hewson observed that blood corpuscles were destroyed in water but remained normal in salt solution. A century later came the famous experiments of Ringer from which resulted the now much used Ringer solution.

Two points of view have guided investigators in the selection or preparation of solutions in which to immerse tissues; the one has been along physical and the other along chemical lines. When blood corpuscles are to be studied outside the body, there is no thought of supplying a nutritive solution but only of supplying one that will prevent a collapse in the normal condition of the cell, such as hemolysis (the loss of the hemoglobin by dispersion through the membrane). But when cells are to be grown in laboratory cultures, as are animal tissues or plants, then the first prerequisite is nutrition; physical factors, such as osmotic pressure, receive secondary consideration. Salts may

thus be discussed from these two viewpoints—first as nutritive media, then as physical systems.

Possibly the first salt solution made for growing plants in the laboratory without soil is that of the German botanist Sachs, followed by the now better known one of Pfeffer. The latter contains

Ca(NO ₃) ₂	0.8 gram	KH ₂ PO ₄	0.2 gram
KCl.....	0.1 gram	FeCl ₃	Trace
KNO ₃	0.2 gram	Water.....	1 liter
MgSO ₄	0.2 gram		

Knop's solution for plant cultures is similar; it contains the same compounds in the same proportions except that potassium chloride is omitted and ferric phosphate is substituted for ferric chloride. The American plant physiologist Shive has developed the simplest of nutrient solutions; it contains but three salts—calcium nitrate, potassium phosphate, and magnesium sulphate, besides the usual trace of iron; it thus supplies the seven necessary elements (in addition to carbon, hydrogen, and oxygen, which are obtained from water and air).

The almost perfect nutrient fluid for animals, at least for the young of the same species, is milk. Except for its low iron content, it contains the elements essential to animal growth. Wright and Papish made analyses of milk using the spectrographic method. A spectrographic record was obtained of the dry matter in milk by burning and viewing it photographically through a quartz spectrograph. The following elements were found:

Calcium	Iron	Silicon
Magnesium	Copper	Boron
Potassium	Zinc	Titanium
Sodium	Aluminum	Vanadium
Phosphorus	Manganese	Rubidium
Chlorine	Iodine	Lithium
		Strontium

The first six occur in large quantities; the remainder, only as traces. Chlorine and iodine could not be confirmed by the spectrographic method, but their presence had been adequately established by other methods.

This list, with a few additions, may be regarded as representing the essential elements for animal life. Sulphur should be added,

as it too has been found in milk. It is supplied to the adult by protein foods and is either used in increasing the protein content of the body and repairing protein waste or oxidized for the production of energy and lost as sulphate in the excreta.

L. G. Wesson suggests the following salt mixture for use in compounding synthetic diets for experimental animals:

Mixture	Grams	Mixture	Grams
NaCl.....	105.0	FePO ₄ + 4H ₂ O.....	14.7
KCl.....	120.0	MnSO ₄ (anhydrous).....	0.20
KH ₂ PO ₄	310.0	K ₂ Al ₂ (SO ₄) ₄ + 24H ₂ O.....	0.09
Ca ₃ (PO ₄) ₂	149.0	CuSO ₄ + 5H ₂ O.....	0.39
CaCO ₃	210.0	NaF.....	0.57
MgSO ₄ (anhydrous).....	90.0	KI.....	0.05

This mixture supplies the following ions: sodium, potassium, calcium, magnesium, iron, manganese, copper, aluminum, phosphate, sulphate, chlorine, fluorine, iodine.

It must be remembered that salts supply only the inorganic needs of animals. An organic (carbohydrate, fat, and protein) diet is the first prerequisite for an animal; the salts merely supplement it. (The claim has been made, notably by S. O. Mast, that certain simple animals, such as *Chilomonas paramecium*, can exist on a purely inorganic diet.)

Salt solutions exercise certain influences upon protoplasm because of their osmotic, electric, or other purely physical properties, considered apart from their chemical constitution (it is always very difficult wholly to dissociate the two). Ringer found that sodium chloride used for the continuous perfusion of the heart of the frog removed from the body does not maintain the normal beat. This may be due to the lack of some other salt, essential because of itself alone or because it is needed to counteract a possible "poisonous" effect of the sodium chloride. The osmotic pressure of the solution may also be at fault. This last possibility was taken care of in Ringer's experiments by making the solution isosmotic with (of the same osmotic value as) blood (which is given by 0.75 per cent sodium chloride). The two other possibilities Ringer proceeded to study. He found that calcium chloride added to the sodium salt caused the heart to renew beating, although diastole (dilation) was imperfect. Calcium augments systole (contraction). Ringer next discovered that potassium has an effect opposite to that of calcium; it

accelerates diastole and thus nicely counterbalances calcium without robbing it of its beneficial effects. The solution was further slightly improved by the addition of a small amount of sodium bicarbonate, which produces a slight alkalinity similar to that existing in the blood and maintains this alkalinity (like a buffer) by neutralizing the acid produced by the heart muscles in contracting. Thus resulted the now justly famous Ringer's solution the composition of which is as follows:

	Parts		Parts
NaCl.....	6.5	NaHCO ₃	0.2
KCl.....	0.14	Na ₂ HPO ₄	0.01
CaCl ₂	0.12	Water.....	1,000

Such a solution is said to be *physiologically balanced*.

Magnesium is omitted from Ringer's solution though it is put in all artificial plant nutrients and occurs in sea water. The following two lists give the salts found in the ocean and those which make a good imitation sea water for laboratory work:

Ocean (To 1,000 parts)	Artificial (To 1,000 cc. of distilled water)
Grams	Grams
NaCl.....	26.86
KCl.....	0.58
MgCl ₂	3.24
MgSO ₄	2.20
CaSO ₄	1.35
Rest.....	0.07
	(NaHCO ₃ as a buffer).....

There is a surprisingly close agreement in the proportions of the three important elements sodium, potassium, and calcium, in Ringer's solution and in a number of natural solutions which serve as a medium for maintaining life. How striking this is is to be seen from a comparison of the quantities of these elements in three physiologically balanced solutions:

Element	Ringer's	Sea water	Blood serum
Na.....	100.	100.	100.
K.....	2.15	3.66	6.69
Ca.....	1.85	3.84	2.58

It has been suggested that the salt of the blood of land vertebrates is an inheritance from marine ancestors. When verte-

brates left the sea for the land, they took with them a blood system which was in equilibrium with the sea water in which it had developed. The following table (from Henderson) compares the relative proportions of salts in the ocean and in vertebrate blood:

Element	Sea water	Blood serum
Na.....	30.59	39.0
Mg.....	3.79	0.4
Ca.....	1.20	1.0
K.....	1.11	2.7
Cl.....	55.27	45.0
SO ₄	7.66	
CO ₃	0.21	12.0
Br.....	0.19	
P ₂ O ₅	0.4

The differences in salt concentration are explained by differences existing between the salt content of the sea when our vertebrate ancestors were marine and the salt content of the sea today. The vertebrates took with them the proportions of salt existing in the sea at the time when they left it. Since then, the sea has changed, but not the blood.

Whether life started in the sea or on the land we cannot say; but if on land, it soon went into the sea and remained there until the close of the Cambrian period. In early Cambrian, perhaps, the blood stream was established. Our blood is, therefore, presumably as salty as that of the ocean in early Cambrian, and it is one-third as salty as is the sea at present.

Substitution.—The question whether or not some other element will do as a substitute cannot, as we have seen, hold in certain cases (*e.g.*, barium for calcium). The answer for boron has been given by Brenchley, and it too is No. Among 52 elements tested, none proved capable of replacing boron. McHargue says the same for manganese; no other one of the more common elements, including iron, copper, zinc, boron, or arsenic, will replace manganese in the growth of plants. These facts indicate that in nutrition an element plays a certain role because it is *that* particular element and not merely because it

is one of a special group of elements. It is difficult to distinguish between what is chemical and what is physical, yet it may be said that in physical properties (electrostatic effects determined by sign of charge and valency), any element within a group (monovalent, divalent, etc.) will often serve as a substitute for any other in the same group, but in its chemical (nutritional) requirements, an element has no substitute. The plant demands calcium, boron, or manganese and not a near relative of it. Not only in nutrition but also in their effects on protoplasm, substitution among the elements is not always possible. The presence of calcium in the water makes healing of the cell membrane possible owing to the coagulating effect of this element. Magnesium will not replace calcium in this respect. The question of substitution is part of that of antagonism.

Antagonism.—Magnesium is necessary for plant growth, yet it may, when certain other elements are not present in sufficient quantity, produce "magnesium injury." This was investigated by Tottingham and Trelease. The symptoms of magnesium injury of wheat are so conspicuous and distinctive that the disease furnishes a striking example of growth derangement resulting from a disturbed salt nutrition. Trelease has shown that calcium added to the nutrition of a plant in sufficient quantity will prevent the injury caused by magnesium. The occurrence and severity of the injury are determined by the ratio of magnesium to calcium. The prevention of magnesium injury by calcium is an example of what is known as *salt antagonism*. The role of calcium in this case is primarily physical, as the injury is due to magnesium toxicity and not to calcium deficiency. This is supported by the observation that strontium is also able to delay and partially inhibit the characteristic symptoms of magnesium injury. Such instances are ones where substitution among elements of a group is possible.

The antagonistic action of salts has furnished an interesting and little understood chapter in physiology. It was discovered by Ringer when he observed that the addition of calcium to a common salt solution made the latter a better fluid for the perfusion of the heart, not only because the element calcium was needed by the tissue but also because a partial toxic effect of the pure sodium was counteracted. J. Loeb continued the study of antagonism.

Loeb was inclined to think of elements as physical rather than as chemical entities. For him, calcium played its role in life because it is bivalent and because it possesses two electrical charges, rather than because it is the element calcium. If Loeb's viewpoint is true, then any other bivalent element with two electrical charges should be able to replace calcium as an antidote. Experiments by Loeb prove that out of six bivalent elements, five antagonize sodium equally well. The experiments had to do with the percentage of *Fundulus* eggs that develop into embryos in solutions of various salts. It must be remembered that the six elements are alike in but one respect (in the experiments under consideration), *viz.*, that they all antagonize sodium. This does not mean that they can in all other respects replace each other in the life of an organism.

Solution	Percentage of Eggs That Develop into Embryos
100 cc. $\frac{5}{8}$ M. NaCl.....	0
100 cc. $\frac{5}{8}$ M. NaCl + 2 cc. 1 M. BaCl ₂	90
100 cc. $\frac{5}{8}$ M. NaCl + 2 cc. $\frac{5}{16}$ M. SrCl ₂	90
100 cc. $\frac{5}{8}$ M. NaCl + 2 cc. 1 M. MgCl ₂	75
100 cc. $\frac{5}{8}$ M. NaCl + 2 cc. $\frac{1}{8}$ M. CaCl ₂	88
100 cc. $\frac{5}{8}$ M. NaCl + 4 cc. $\frac{1}{8}$ M. NiCl ₂	5
100 cc. $\frac{5}{8}$ M. NaCl + 8 cc. $\frac{1}{128}$ M. ZnSO ₄	75

For Loeb, then, antagonism became simply a question of the opposing influence of monovalent elements on the one hand and bivalent elements on the other. As an antidote one element was as good as another in its own group. R. Lillie found that while practically any bivalent cation will annul or diminish the toxic influence of sodium chloride, the efficiency of the elements in this respect put them into the following decreasing series (in which $M/2$ NaCl was mixed with $M/200$ of the bivalent metal): Mg > Ba > Ca > Sr > Mn > Fe > Co > Ni > Cd > Pb > Zn > Cu. (It is interesting that the last three strongly toxic elements copper, zinc, and lead should antagonize, *i.e.*, prevent the poisonous action of, the relatively harmless salt sodium chloride. This becomes more comprehensible when it is realized that the concentration of the sodium is one hundred times as great as that of the heavy metals.)

There are other serious objections to the valency grouping of ions from the physiological point of view. Monovalent cations antagonize each other, and so do bivalent ones. Rubinstein found that the toxicity of sodium manifests itself in a number of ways and that calcium will counteract it in one way only, while potassium counteracts it in still another way. Here two monovalent elements (sodium and potassium) and a monovalent and a bivalent element (sodium and calcium) antagonize each other. Stover found that mice eating food containing a mixture of calcium carbonate and barium carbonate (a poison) are unharmed. Here bivalent metals (calcium and barium) antagonize each other. The results of Trelease were similar, the antagonism being between two bivalent ions. Gellhorn found that calcium decreases the fatigue of muscles, while barium (and strontium) increases it; combined, the elements have no effect. Thus does calcium antagonize barium, although each is bivalent. The action is specific in that the calcium cannot be wholly replaced by magnesium. There is also a slight antagonism between the two monovalent ions sodium and potassium.

We are dealing here with two facts both of which oppose the valency grouping of ions in physiological processes; first, while monovalent ions (sodium) are opposed by bivalent ones (calcium), it is equally true that mono-valents (sodium) oppose mono-valents (potassium) and that bi-valents (calcium) oppose bi-valents (barium). Furthermore, not every member of a group will do as an antagonist; thus, magnesium will not replace calcium. This last fact is further supported by the work of Pantin, who found that calcium, in certain cases, can be replaced by strontium but not by magnesium or barium.

The nature of salt antagonism is unknown. It was first thought that the ions opposed each other in solution, that is to say, that the antagonism was outside the cell. One might imagine two opposing armies meeting on a field of battle outside the city walls, each preventing the other from entering and doing damage. But this hypothesis has little to support it. More reasonable is the possibility that ions exhibiting antagonistic action exercise an opposite effect on protoplasm. If sodium produces one effect upon protoplasm and calcium an opposite effect, then these two elements in proper proportion should leave the protoplasm unchanged. This they apparently do in

a physiologically balanced solution, where the proportion of sodium to calcium is 50: 1 or 2, as it is in blood and sea water. If we accept this hypothesis, the question arises, Just which property of protoplasm is it upon which the two ions have opposite effects?

The effect of sodium and of calcium on protoplasm is a controversial subject, though the consensus of opinion appears to indicate that, in general, sodium disperses and calcium aggregates. The work of Osterhout showed that sodium increases and calcium decreases protoplasmic permeability (though Fitting found that potassium, which one would expect to act like sodium, decreases permeability; and there were also qualifications in regard to calcium in Osterhout's work). The work of Ruth Addoms indicates that sodium, potassium, calcium, magnesium, zinc, and aluminum salts all coagulate the protoplasm of root hairs. But the experiments of Fauré-Fremiet and of Plowe support our original and rather generally accepted contention that protoplasm thickens in the presence of calcium and exhibits extraordinary elastic qualities; while in the presence of sodium it is less thick and poorly elastic. There is the further experimental evidence that a torn protoplasmic surface is repaired only in the presence of calcium, owing to the aggregating (gelating) effect of the latter, an effect that sodium lacks. This action is further indicated by the fact that when calcium is injected into a cell, the micropipette may be removed immediately without danger of loss of protoplasm, due to the rapid coagulation of the protoplasm by the calcium at the point of injection. With sodium, this is never true, and the pipette cannot be withdrawn immediately or quickly. Freundlich, thinking of the action of salts upon colloidal systems in general, suggests that the colloidal material in protoplasm is precipitated or aggregated by the calcium ion and dispersed by the sodium ion. Höber found this to be true in the case of proteins. Gellhorn states that calcium reduces the imbibition qualities of protoplasm and that sodium augments them.

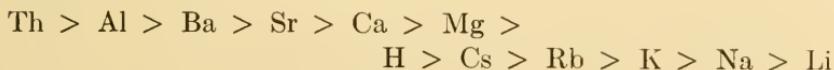
If the opinion of the majority holds, then it seems probable that the antagonistic action that sodium and calcium exhibit is due to the dispersing (liquefying) effect on protoplasm of the former and the aggregating (coagulating) effect of the latter. The antagonistic action of these two elements as shown by their

opposite effects on the viscosity, permeability, imbibition, and elasticity of protoplasm and its capacity to repair its surface, explains the nontoxicity of these two elements when in proper (50:1 or 2) proportion.

Such, in its simplest form, is salt antagonism. The situation is complicated, as we have seen, by the fact that monovalent elements often show antagonism between each other, that bivalent elements do the same, that not all monovalents or all bivalents have a like effect, and, further, that anions (OH^-) sometimes antagonize cations (Ca^+). The factor so often neglected in such discussions is the protoplasm itself. Protoplasm is in a constant state of change; no two masses of it are ever identical, nor is one mass quite the same during any two periods of time.

While salt antagonism has been studied primarily in connection with protoplasm, it is also involved in simple nonliving systems, *e.g.*, lecithin.

The Lyotropic Series.—The series of elements arranged by Lillie (above) in decreasing order of their antagonistic action on sodium is a *lyotropic*, or *Hofmeister*, series. Many such series have been arranged on the basis of the effects of ions on protoplasm, blood, proteins, and colloidal suspensions. If cations are arranged in the order of their power to precipitate colloidal suspensions (of metals, dyes, or oils), the following series is obtained:



which means that thorium is the most and lithium the least effective in precipitating a suspension, *e.g.*, of an arsenic salt. Two facts stand out—first, that no two ions have the same precipitating power (except possibly caesium and rubidium, which differ but slightly); and, second, that there is a definite arrangement into groups of like valence; the first element is quadrivalent, the second trivalent, the next four bivalent, and the last six monovalent.

The original series arranged by Hofmeister is: Citrate > tartrate > sulphate > acetate > chloride > nitrate > chlorate. It expresses the relative effects of anions (of sodium salts) on the salting out, or precipitation, of egg albumin. Höber found the

same series to hold for the precipitation of lecithin. Pauli gives a similar series based on the influence of acids on the viscosity of albumin from blood: Hydrochloric > monochloracetic > oxalic > dichloracetic > citric > acetic > sulphuric > trichloracetic. The strong monobasic (monobasic, dibasic, etc., express the valence of the anion of the acid) hydrochloric acid increases the viscosity of blood most. It is followed by the weak monochloracetic acid, and this by the dibasic oxalic. Later comes the weak tribasic citric acid, which is followed by the weak monobasic acetic, and this in turn by the strong dibasic sulphuric, which joins another monobasic acid, trichloracetic. The series shows no relationship to valence.

Jacques Loeb, antagonist of colloidal phenomena which appear to run counter to classical physicochemical rules as do the Hofmeister series, flatly denied the existence of such series. He stated that they are purely fictitious and due to a failure to measure the acidity of the solutions. The effect, Loeb maintained, is the result of a change in acidity which the ions produce; that is to say, an ion is effective within its valence group only in so far as it alters the acidity of a solution. If the solutions are all brought to the same acidity value, the effects of the ions will be of four degrees only, caused by mono-, di-, tri-, and quatravalent ions. Loeb then proceeded to prove this. He took a typical Hofmeister series, such as is claimed to show the relative effects of anions on the osmotic pressure, swelling, and viscosity of protein solutions: $\text{SO}_4^- < \text{acetate}^- < \text{Cl}^- < \text{Br}^- < \text{NO}_3^- < \text{I}^- < \text{CNS}^-$. The particular property that Loeb measured was the potential at the surface of a collodion membrane. He used sodium salts of all of the anions in the above series and found that if the solutions are kept at the same acidity value, the anions each produce quantitatively the same depressing effect on the membrane potential, except for sulphate, which is the only bivalent anion in the group. (The depressing effect of sodium sulphate is much greater.) It must be admitted that there is some truth in Loeb's argument. His experiments reduce the Hofmeister series simply to quatrivalent > trivalent > bivalent > monovalent ions.

It is certainly true that large valence effects overcome lyotropic ones; most of the latter are within a valence group; but, while acidity is a significant factor in protein solutions, this is not true

of salt solutions, where excellent lyotropic series result, and pH cannot possibly be a factor; thus, the surface tension of salts gives the following definite lyotropic series: $\text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^- > \text{SCN}^-$.

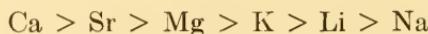
If the situation is viewed as physiologists would see it, it would be necessary to grant, on the basis of Loeb's contention, that the effect of ions is not specific but depends solely on electrostatic properties such as valence and sign and magnitude of charge; in this case, a plant or an animal should be indifferent to the ion it receives just so long as it is of the proper valence and charge. Were this true, then our entire story of ion nutrition would collapse. Any monovalent ion or any bivalent ion would do, which we have already seen is not true. A plant needs potassium and will have little or nothing to do with sodium, while an animal demands sodium, and potassium will not do as a substitute. (The heavy metals, which in any but the smallest quantities are highly toxic, Loeb excepted. They and hydrogen do not come into the valence rule.) Northrop summarizes the valence rule as applied to physiological and similar organic reactions, as follows: There is no doubt that any individual effect of ions of the same valence—except for hydrogen and heavy metals—is so small in comparison with the pure valence effect as to be entirely negligible. If precautions in regard to acidity are taken, a great many of the Hofmeister series disappear. However, a general statement cannot be made to cover all types of experiments. In concentrated salt solutions and in respect to other properties or other experiments, there is very likely a definite characteristic ion effect. (Loeb's experiments were limited to fairly dilute salt solutions.) This conclusion deserves serious consideration and undoubtedly holds for metal suspensions—where the effect of ions is in direct relation to valence and electric charge, though not in direct proportion, as tri- and quatravalent ions are more than three and four times as effective as monovalent ones. However such a conclusion is not generally held in regard to protein solutions of which the Hofmeister series (with pH control) are very characteristic.

That Hofmeister, or lyotropic, series are more characteristic of protein than of metal suspensions is due to the fact that hydration is a greater factor in the former. Kruyt states that lyotropy is an expression of hydration, pure and simple, of the orientation of water dipoles, which explains why, in proteins, the

lyotropic series are reversed when the charge of the protein changes from positive to negative.

Lyotropic series of ions occur not only in regard to the effects of ions on colloidal solutions but also in regard to the hydration of other ions. Ions carry water molecules along with them; this is an important factor in determining their migration rates. They become grouped into a typical lyotropic series when arranged in order of their hydration effects on other ions. Such a series expresses the order in which the ions take up water or affect the dissociation of water. Kruyt emphasizes the fact that hydrogen ions cannot possibly be the sole cause of lyotropic series.

While a lyotropic, or Hofmeister, series is typical of protein systems, valence may be equally evident. This is true of protoplasm; thus, work by Plowe on the extensibility of protoplasm shows that while ions are grouped according to valence, there is a pronounced difference among the individuals of a group; and that while the hydrogen ion (acidity) is a factor, it is not the only factor. Protoplasm was stretched by microneedles and the length of the thread measured. The following grouping of ions resulted:



which means that protoplasm is poorly elastic in sodium salts and highly so in calcium salts. While all the divalent ions are together, calcium increases the elastic qualities of protoplasm to a great degree, while magnesium has no noticeable effect whatever. The behavior of protoplasm when in an aluminum salt (not included in the series) illustrates the relative effect of a metal as such and of the change in acidity that it occasions. Aluminum is known to increase the acidity of solutions. One must, therefore, ascertain how much of the effect caused by an aluminum salt is due to the aluminum ion itself and how much to the increased acidity. In order to do this in the preceding experiment, a solution of hydrochloric acid of the same pH value as the aluminum solution was added to the cells. In this way, it was ascertained that four-ninths of the increase in stretching capacity of the protoplasm produced by an aluminum salt is due to the aluminum ion itself and five-ninths to the increase in acidity. Thus it is shown that part of an apparent ion effect may be due

to acidity alone, but also part to the ion itself. Further evidence lies in the fact that the solutions of the sodium, magnesium, lithium, and calcium ions were all of the same acidity, yet there was a pronounced difference in the effect of these solutions on the stretching capacity of protoplasm.

Loeb did well in calling attention to an experimental error, but experiment proves and opinion upholds a specific ion effect. It would be difficult to escape this conclusion in the face of the definite need of organisms for certain ions and the marked preference shown by plants and animals for ions that are physically as closely related as are sodium and potassium or calcium and magnesium.

Organic Salts.—While this chapter is concerned primarily with inorganic salts, brief reference may be made to certain organic salts which may play an important role in protoplasm, *viz.*, the soaps. A soap is a metallic salt of a higher fatty acid, usually an alkali metal united to oleic, stearic, or palmitic acid; thus, sodium stearate is $\text{NaC}_{18}\text{H}_{35}\text{O}_2$. Soaps are minor ingredients of protoplasm, small in quantity but possibly exercising considerable influence. They possess some of the properties of crystalloids, in that they form crystalline structures and ionize; and of colloids, in that they form elastic jellies. One possible role of soaps in protoplasm is the saponification of fats and the maintenance of the protoplasmic emulsion.

Biological and Medical Problems in Salt Concentrations.—Life is the expression of the interrelationship between protoplasm and its environment. In the composition of this environment, salts play a part second only to that played by water. The salt balance between a living cell and the solution that bathes it must (ordinarily) be accurately maintained, or else illness and death result. There often appear to be striking exceptions to this, but usually they are only apparent exceptions. Thus, Mast finds that Amoeba without food moves normally, divides, and lives several (a maximum of 16) days in water of very high purity. Salts in proper concentration, both single and mixed, prolong the life (a maximum of 18 days in single salts and 22 in mixed ones). The experiment does not reveal the ill effects of the absence of salt while the animal lived.

Organisms sometimes show a remarkable adjustment to a change in salt environment. The most surprising examples of

this are certain crabs. The little red fiddler crab, *Portunus depurator*, when put into diluted sea water rapidly changes the concentration of its body fluids, presumably by transfer through its gills, until, in a few hours, it has come into equilibrium with its new surroundings. If the change is not too great—say, to 75 per cent sea water—it will live; but in 50 per cent sea water, it dies. The shore crab, *Carcenus maenae*, lives where it must be ready to meet diluted sea water in estuaries or concentrated sea water in pools, from one to the other, day after day. It can tolerate 25 per cent sea water for a long time, slowly diluting its body fluids until they reach a value isosmotic with about 60 per cent sea water. The gills are presumably permeable to the salts of sea water but can also resist their passage. They are able to tolerate a considerable difference of concentration on their two sides—a property essential to the crab's existence and probably very important to some of our own cells, e.g., the kidneys and alimentary canal.

In higher animals, the absence of salts is made evident quickly and strikingly. Miners' cramp is an example. Miners, in common with other workers in hot atmospheres, perspire profusely, losing sometimes $\frac{3}{5}$ lb. in weight per hour. If this loss is replaced by drinking pure water in quantity, cramps result. These may be prevented by the addition of a very small quantity of salt to the water. The explanation from the point of view of salt equilibrium is obvious. Perspiration contains much salt. Replacing the loss by water alone disturbs the natural balance between the blood and the tissues; the latter, being left in a too acid condition, swell, just as does acidified gelatin, and cramps result. The presence of a neutral salt in the water prevents this.

Salt concentration and salt balance have become important subjects in medical practice. The salt content of the blood is of diagnostic value. Proper bone formation depends upon a proper calcium-phosphorus equilibrium.

The value of salts or their metals is coming more and more to the fore in dietetics. We are told that whole wheat, unpolished rice, baked potatoes, etc., are better food not only because vitamins are in the husk and skin but because biologically important metals are also there. Plants store small amounts of copper, manganese, and zinc in the husks and embryos of their

seeds. When corn, wheat, and rice are highly milled, the polished, or "degermed," corn meal, patent flour, and rice are deprived of the greater part of the compounds of copper, manganese, and zinc, which are factors in animal nutrition. In agriculture, some depleted soils require the addition of small amounts of compounds of copper, manganese, and zinc in order to restore and maintain productivity through the supply of necessary vital elements.

Pernicious anemia was believed to be due to a shortage of iron. Milk is deficient in iron, yet hemoglobin is rich in this metal. It was, therefore, assumed that the way to correct anemia would be to add iron to the milk diet. In the case of animals, this plan proved ineffective. The daily feeding of iron, administered as chloride, sulphate, acetate, citrate, or phosphate in the milk, all prepared from pure iron wire, did not check a decline in hemoglobin content of the blood. Rats suffering with anemia were not improved. But when they were fed dried liver, the ash of dried liver, corn, or lettuce, which contain iron and copper, the hemoglobin was raised to normal, and the stricken rats immediately restored to health. E. B. Hart found that when copper sulphate is added to pure iron chloride in the whole-milk diet, cures result. Rats, so anemic that their days appeared to be numbered, recovered immediately, and the hemoglobin in their blood was brought to normal.

The role of copper in animal life is probably similar to that of iron in plant life. Hemoglobin does not contain copper, and chlorophyll does not contain iron, yet copper promotes the building of hemoglobin, and iron promotes the production of chlorophyll. Both function as catalysts.

The distribution of salts in the body is not uniform. Concentration and similar gradients result. A salt gradient must inevitably mean an osmotic and an electric potential gradient and is strong evidence of a metabolic gradient. The conductance (page 337) of samples of plant sap squeezed from tissue at several points along the plant should indicate a gradient if one is there. Hurd-Karrer used specific gravity as an indicator and found that the juice of corn stalks taken from successive internodes showed a progressive increase in specific gravity from the ground up. Similar salt gradients have been reported in trees by a number of workers.

Some interesting experimental results are obtained from the toxic effects of salts on protoplasm, particularly in reference to protoplasmic streaming. Trace elements are highly toxic in slightly higher than normal concentrations and may stimulate protoplasm to an excessive rate of activity. A trace of copper or weak concentrations of barium and strontium chloride will arouse Elodea leaf cells to an excessively high rate of streaming.

The appearance of unexpected applications of purely theoretical discoveries which prove to be of considerable economical value is the rule in science. Such a one is the use to which Nägeli's discovery of the "oligodynamic" effect of copper has been put. Cress growers found their cress dying from some unknown cause, which proved to be smothering by the green alga Spirogyra. The investigator in charge, recalling Nägeli's classical experiment, added copper in the concentration of 1: 50,000,000 parts. The Spirogyra was destroyed, the cress unharmed, and the cost negligible. Pathogenic bacteria in drinking water and mosquito larvae in stagnant water may be killed in a similar way.

It is evident that salts play an important role in life. But *how* important? Do they determine not only the state of our health but perhaps also what we are? One of the most fundamental concepts in biology is the relative stability of protoplasm. Protoplasm is thought of as being a very stable substance, unalterable in its ultimate nature and course of development, yet C. R. Stockard has produced changes in fish development, such as double monsters, and J. Loeb brought on the parthenogenetic development of an egg (*i.e.*, without fertilization) simply by a change in the salt content of the surrounding solution. How much of us, then, is what it is because of our particular kind of protoplasm, and how much because of our external or internal salt environment?

CHAPTER XXIII

CARBOHYDRATES

The carbohydrates, so called because they are built up of carbon and water, are a varied group including such diverse substances as sugar, starch, cellulose, agar, and vegetable gums. The mere statement that the sugars are the chief source of energy liberated through respiration in plants and animals, that starch is an important form of stored energy in the plant and source of energy in animal food, and that cellulose constitutes the framework of higher plants is sufficient to indicate the great role played by the carbohydrates in organisms. Food is translocated (shipped) in the plant primarily in the form of sugars, and the osmotic value of cells is maintained chiefly by sugars. Among the constituents of protoplasm, sugars are exceeded in importance only by water and the proteins.

The carbohydrates are classified as follows:

I. Sugars

A. Monosaccharides, or simple sugars

(Formaldehyde, CH_2O , is the first homologue of the series, but it is not a sugar)

1. Pentoses, $\text{C}_5\text{H}_{12}\text{O}_5$ —arabinose.

2. Hexoses, $\text{C}_6\text{H}_{12}\text{O}_6$ —glucose or dextrose (grape sugar), fructose or levulose (fruit sugar), galactose, mannose.

B. Disaccharides, or compound sugars, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ —sucrose or saccharose (cane sugar), maltose, cellobiose, lactose (milk sugar).

C. Trisaccharides, $\text{C}_{18}\text{H}_{32}\text{O}_{16}$ —raffinose.

II. Nonsugars

A. Starches ($\text{C}_6\text{H}_{10}\text{O}_5$)_{*x*}—starch, dextrin, glycogen.

B. Gums ($\text{C}_6\text{H}_{10}\text{O}_5$)_{*y*}—vegetable gums, mucilages, pectins, pentosans ($\text{C}_5\text{H}_{10}\text{O}_5$)_{*y*}.

C. Cellulose ($\text{C}_6\text{H}_{10}\text{O}_5$)_{*z*}—normal (α) cellulose, lignocellulose.

The examples given in any one group in the above table have the same basic formula for that group, but they may differ greatly in other respects; thus, glucose and fructose both have

the formula $C_6H_{12}O_6$; both have the same molecular weight; and both would, on analysis, yield 40 per cent of carbon, 6.6 per cent of hydrogen, and 53.3 per cent of oxygen, yet they taste different and have different optical properties. These differences are due not to chemical composition but to structure. The individuals are said to be *isomers* of each other. The structural isomerism responsible for the difference is illustrated in glucose, which has a CHO group and is known as an aldehyde (or aldose sugar), while its isomer fructose has a CO group and is known as a ketone (or ketose sugar).

The pentose sugars, with five carbon atoms, are not often found free in nature; they are usually associated with mucilages, such as the pentosans. They seem to be of some physiological importance, since they always occur with nucleic acid which constitutes a large part of nuclear material.

Glucose and fructose are the only two common and free hexose sugars. The rest occur associated as constituents of higher compounds such as the glucosides, an important group of substances among which are saponin and digitalin. Glucose is the most widely distributed of the sugars and is the energy-producing sugar oxidized in the bodies of higher animals—probably also in those of the lower animals and in plants. It is the universal fuel sugar, which means that other sugars when taken in as food are ultimately converted into glucose before being oxidized or broken down into carbon dioxide and water. Glucose occurs in blood to the extent of about 0.14 per cent. It is abundant in fruits; the brown powder on the surface of raisins consists of glucose crystals; molasses is one-third glucose. Honey contains glucose, but the chief constituent here is fructose, the sweetest of sugars. It is usually found wherever glucose occurs. Glucose is commercially prepared from starch, the following series of changes taking place: Starch \rightarrow dextrin \rightarrow maltose \rightarrow glucose. The importance of sugars as food has led to attempts to produce them from cheap raw material such as sawdust. This seems to be already a commercial process in Germany. Work done at the Forest Products Laboratory in Madison, Wisconsin, suggests that the method may be some day widely used. It appears that cellulose (wood) may, by hydrolysis, be converted into sugar; that is to say, by combining water chemically with cellulose, glucose results.

Galactose is an important sugar; as part of the lactose molecule, it becomes part of the carbohydrate food of the nursing animal. It is also found in brain and nerve tissue generally.

The disaccharide sucrose is the sugar of our daily life. It may be cane sugar, or beet sugar, as in France. Sucrose is of very wide distribution in the plant kingdom but not, in the same sense, in the animal kingdom, where it serves as food to be broken down into simple sugars. It is interesting that honey is almost wholly "invert sugar" (a mixture of glucose and fructose), yet the bee collects cane sugar (sucrose) from the flowers. The hydrolytic agent acting as catalyst in this reaction is probably the formic acid secreted by the bees.

The first synthesis of a sugar was done by Emil Fischer. More recently, the French-Swiss chemist Pictet reports the artificial synthesis of sucrose in his laboratories in Geneva.

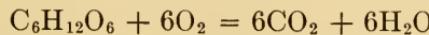
Maltose and cellobiose are less common plant disaccharides. Lactose is found in milk and nowhere else. It is a constituent of the milk of all mammals; it is the sugar for the nursing young.

Among the trisaccharides, raffinose is the best known. While its presence has been reported in certain invertebrates, it is almost solely a plant sugar.

In addition to their nutritive qualities, sugars come into play in organisms in the generation of such pure physical forces as osmotic pressure. The sugar content of plant vacuoles is the chief factor involved in turgor.

Because sugars are readily soluble in water, it is probably in this form that most food is translocated, or "shipped," in plants and animals. Digestion consists in the breaking down of higher foods into a diffusible or assimilable form. Food in plants is stored as starch, fats, or protein but shipped as sugar. When the sap flows in the spring, it is sugar that is sent up to the young opening leaves.

Sugars play their chief role in organisms as the source of energy released in respiration. They are reduced by oxidation to their simplest member glucose, the energy stored in them being thus liberated; carbon dioxide and water result:



Other substances are oxidized (notably the fats), but, as R. B. Harvey states, from the standpoint of the quantity of energy

transformed in the living world, the simple sugars are the key substances concerned. Respiration is, however, not the only physiological process in which the sugars play a prominent part. Muscular action involves glucolysis, or the conversion of glycogen (starch) into glucose; and glycogenesis, the return from glucose (or lactic acid) to glycogen. The body utilization of the lower carbohydrates is an important problem in the study of cancer. The total blood sugar is high in cancer and in diabetes, but the utilization of this sugar (oxidation to lactic acid) is rapid in cancer, with attendant rapid cell growth, while it is sluggish in diabetes.

Starches.—The higher carbohydrates—the polysaccharides or nonsugars—are mostly insoluble in water, but they take up water readily and form pastes and jellies; they are therefore colloidal.

The starches are sometimes referred to as amyloses and, together with the celluloses, as hexosans, because on hydrolysis they yield hexose sugars:



Starch is one of the most widely distributed of substances in the vegetable kingdom; it is the chief storage food of plants and may constitute 70 per cent of the dry weight of seed. The structure of the starch grain, as it occurs in the plant, is very characteristic and is used as a means of identification. Its chief distinguishing feature is its layered or lamellar structure (Fig. 11). Starch is of biological importance because of its nutritive qualities, its extraordinarily high imbibition pressure, and its paste-forming qualities. Whether or not the imbibition pressure of starch is in part responsible, as has been maintained, for the carrying of water to the tops of trees cannot be said, but it certainly plays a part in bringing water into the cell. The gelatinous properties of starch may, to a great extent, be responsible for the highly viscous properties of protoplasm. Starch paste has some of the properties of a true elastic jelly and some of those of a plastic mass (page 227), but much of the viscous, glutinous, and elastic properties that one might be inclined to attribute to starch, *e.g.*, in such substances as bread dough, are in great measure due to associated matter. Gluten comprises 10 per cent of wheat.

When flour is freed of starch, gluten remains behind as a tenacious, sticky mass. It is less abundant in foods than is starch but an equally valuable foodstuff.

Another amylose is dextrin; it is an intermediate product between starch and glucose. Some of the so-called "soluble" starches are probably dextrins. They are not abundant in plants. Dextrin is used as a substitute for gum.

Glycogen, or animal starch, occurs rarely in plants—in only a few of the fungi. It has risen to great prominence of late as the fuel for muscular action, though it has long been recognized as a substance of great physiological importance, especially in the liver where formerly it was thought to exist simply as stored excess carbohydrate but now is viewed dynamically, that is to say, as fuel for energy. (The distinction is probably not great, and glycogen is still stored in part as food.) It is readily converted into the soluble sugars maltose and glucose, by the starch (amylum)-splitting enzyme amylase.

Muscular Action.—The search for the source of energy in the contraction of muscle dates back nearly a century, when Helmholtz concerned himself with the relation between heat production and muscular energy.

The names of three Englishmen and one German are intimately associated with our present knowledge of muscular action—F. G. Hopkins, W. M. Fletcher, A. V. Hill, and O. Meyerhof. The first two investigators found that muscle when stimulated accumulates lactic acid, $\text{CH}_3\cdot\text{CH}(\text{OH})\cdot\text{COOH}$ (so named because it occurs in sour milk). The lactic acid is formed from glycogen, and the amount produced is proportional to the amount of work done. Like a run-down electric accumulator, the exhausted muscle requires recharging. This is done during the period of rest or recovery. The accumulation of lactic acid, which possibly has a toxic effect, causes the muscles to become "tired." (Exhaustion of muscle is not fully comparable to that of an electric accumulator, because in the latter, though there are "poisoning" effects, the stored energy is actually used up; while in muscle, the supply of glycogen is not yet gone even at complete fatigue.) During recovery, which is the period of rest—of recharging or reactivation—oxygen is absorbed, and part of the lactic acid is oxidized and part converted back into glycogen. The reaction becomes, $\text{Glycogen} \rightleftharpoons \text{glucose} \rightleftharpoons \text{lactic acid}$. Thus, muscle tissue

in doing work derives the necessary energy not from oxidation, as is true in many living and nonliving machines, but from the rapid conversion of the carbohydrate glycogen into lactic acid. The action is an exothermic one; *i.e.*, it proceeds without elimination of heat.

When the fatigued muscle recovers, it recharges its store of energy by reconverting the lactic acid into glycogen, to do which it needs a source of energy; this it derives from further oxidation of some of the carbohydrates. The reaction is a coupled one; that is to say, the synthesis of the glycogen cannot take place except through oxidation of part of the lactic acid. As the latter supplies energy for the former, more glycogen must come from other sources—perhaps out of the surrounding solution by adsorption on to the protein constituents of the muscle.

Such was the story in 1924, when Embden stated and afterward often reiterated that part (now all) of the lactate is formed after the contraction is over. Although it had long been known that phosphorus compounds play a part in muscular action (creatine was known to be present in large amounts), the contention of Embden was not fully accepted until six years later, when Lundsgaard proved that muscle may contract without the formation of lactic acid. Thus is lactic acid removed from the central position that it heretofore occupied in muscular contraction. The initial reaction may be the breakdown either of glycogen or of phosphagen. In the former case, the reaction is apparently immediately followed by the combination of a hexose sugar with phosphoric acid to form an ester. Lactic acid is produced later.

Again it must be said that in giving to carbohydrates the chief role in muscular action, it is likely that other substances, notably the fats, may serve as a source of the energy. Hemoglobin, carnosine, and potassium are also present in muscle with no known role. The present opinion seems to be that any foodstuff may be used for the restoration of energy in muscle.

We have a satisfactory (though not the only) explanation of the chemistry of muscular action, but it tells us nothing of the physical mechanism involved. We may learn all the details of coal combustion and yet know nothing about the machinery of a locomotive. As the mechanics of muscular action involves, possibly, such forces as surface tension, imbibition, and struc-

tural organization, it has been considered under these headings (page 334).

Cellulose.—Cellulose chemists recognize not one substance which is cellulose but a group of substances, the celluloses. Modern research has produced an alpha cellulose which is as near a chemical entity as any cellulose heretofore attained, but while this may be regarded as a definite cellulose, there are others. The naturally occurring celluloses are of three groups—the true, the compound, and the hemi- or reserve celluloses. Among the first, that of the cotton fiber is the purest, being 90 per cent true cellulose. Compound celluloses are true celluloses impregnated with other substances. The hemicelluloses are incompletely developed forms of cellulose and other carbohydrate materials such as araban, xylan, etc. In spite of this apparent variety, it does not appear that the celluloses of the various seed-bearing plants are actually different chemical substances; that is to say, while physical differences (*e.g.*, fiber length) exist, and chemical differences in the constitution of the cellulose of the original wood may exist, the residues, termed cellulose, obtained from different woods are probably identical in chemical structure.

Protoplasm, as it builds the plant-cell wall, simultaneously or subsequently secretes substances that occur either as distinct layers alternating with the cellulose or, more usually, as an impregnation of it. Such substances are lignin, suberin, pectin, and cutin. Old wood is lignified cellulose, and cork is suberized cellulose. Pectin may form distinct layers in the cell wall alternating with cellulose, or it may be separately deposited. In general, pectin compounds impregnate the wall, forming so-called pectocelluloses. Cutin is often a surface deposit and occurs as the waxy coating on glossy leaves and fruits. To be superficially deposited, it must pass through the cellulose wall and in so doing adds to the chemical complex that we call natural cellulose.

The hemi- or reserve celluloses constitute an interesting group which differs structurally from the fibrous celluloses. They are more readily hydrolyzed than the true celluloses and break down into sugars (galactose and pentose) of which they are regarded as the anhydrides and from which they receive their names (galactosans and pentosans).

Associated with cellulose, in a manner similar to that just described for pectin, are numerous other compounds generally regarded, like the hemicelluloses, as derivatives of cellulose. Among them are the gums, mucilages, and gelatinous substances, usually produced during heartwood formation. Their origin and chemical constitution are not well understood.

Cellulose is almost wholly a plant product, yet, like most features used to distinguish plants from animals, it is not an infallible criterion of what is a plant and what an animal. Tunicates and insects are reported to have tunicin in their tests or pellicles. This substance is said to be identical with cellulose.

While cellulose is used primarily by the plant as a material for wall building, it may serve, probably in some modified form, as a reserve food. Cellulose is also food for certain animals which, though lacking the capacity to digest it themselves, are nevertheless able to use it because of their intestinal flora. There is no digestive enzyme in the fermentation fluids of higher animals that will act upon cellulose, nor indeed is any intestinal ferment known that will attack the hemicelluloses, the pentosans, or the galactans, yet these last two carbohydrates certainly, and probably some of the higher celluloses, not only are utilized by animals but form an important part of the dietary of herbivora. This is possible because the digestion of the cellulose is carried out by microorganisms. It is said that the intestinal juices of the horse dissolve 70 per cent of favorable nonlignified cellulose but that the ferments are produced by bacteria or Protozoa. The cow is another example of a higher animal that digests cellulose. In all such cases, the fermentation is done by micro-organisms. The digestion products apparently are not monosaccharides, as one would expect, but carbon dioxide, methane, and fatty acids, the last only being suitable for nutrition. (The use of cellulose as food should not be confused with its important function in diet as bulk, or "roughage," promoting peristalsis.)

The classical example of the wood-feeding habit in animals is that of termites. Intestinal Protozoa make it possible for these insects to live on wood. When defaunated (robbed of their protozoan companions) by heat or oxygen, they cannot digest wood and die from starvation when fed it, but they can then live on rotted wood, that is to say, wood predigested by fungi. If intestinal Protozoa of the same kind as were removed are

returned to the termites, they can again transform wood. This experiment, done by Cleveland, led to the further conclusion that wood-ingesting Protozoa form glycogen by splitting the cellulose into cellobiose and decomposing this, in turn, to glucose, from which they build up glycogen.

The statement that no cellulose-digesting ferment is known to occur apparently holds for higher animals and relatively lowly forms such as the insects. But in more primitive animal types, it is possible that the organism itself may handle cellulose, as is true of certain Protozoa and, possibly, snails, where the ferment may be produced by the snail, although this is not conclusive; the snail, like some of the ungulates, may depend upon bacteria for its cellulose digestion, the bacterial colonies living in the mantle of the snail. The enzyme cellulase, from snails, whether produced by the snail or by symbiotic bacteria, appears to be a very sensitive agent for detecting differences between types and varieties of cellulose through hydrolysis. Karrer has shown this to be true and concludes from it that cellulose consists of two constituents which differ in their behavior toward enzymes.

The digestion of cellulose is not limited to animals. The transformation of cellulose by fungi is well known as the dry rot of wood. The manner in which the germinating tube of fungi spores enters the tissue of the host and the way in which pollen grains penetrate the pistil of flowers on their long journey from the stigma to the ovules have long been problems of interest to botanists. It is possible that the spore sprout or pollen tube secretes a cellulose-digesting ferment at its growing point as it moves forward.

CHAPTER XXIV

FATS

Fats play their important part in the life of the cell and of the organism as a whole through protection (against cold, injury, and drying out) and as an important source of reserve energy. They have a further significant role in cellular activities in that they, in great measure, determine selective permeability. Fats constitute, in animals, the principal and, in plants, an important fund of reserve food, but their value as dietary substances is even greater; they may function as, or be the source of hormones and vitamins. It is in this respect that fats and fatlike substances have received particular attention of late.

The word "fat" will be used here to include not only the fats proper—the fatty-acid tri-esters of glycerol—but also other fatlike substances which are of so much interest to the biologist. Certain of these latter substances are grouped together as *lipoids*. The term is an old one and in common use, but it has met with considerable opposition because it brings together a somewhat diverse group of substances. Substitutes for it, such as lipin and lipide, are no more satisfactory, for they too have been variously used; thus, Bloor recommends "lipide" as a general group name to include the true fats and the lipoids. "Oil" is another term the meaning of which is not definite. It is best distinguished from fat by regarding the simple lipides, *i.e.*, the esters of the fatty acids with glycerol, as oils when liquid and fats when solid. While such a terminology might call for another word as the all-inclusive group term, for which purpose Bloor has suggested lipide, yet until the nomenclature is definitely decided upon, fat will serve better than any other word to convey a conception of the qualities possessed by those substances listed below.

Fats are of three kinds, the simple ones, or true fats, which are esters of the fatty acids with either glycerol (the fats proper) or alcohol (the waxes); the compound ones, which are chiefly the

phosphatides (lecithin and cephalin); and the derived ones, *viz.*, the fatty acids and sterols (cholesterol).

Dietary fats have risen from their former position as mere fuel for the expenditure of energy to the more significant role of a nutritive necessity. Fat components (unsaturated fatty acids such as linoleic acid) are now classed by Burr and Evans as nutritive needs of the same importance as amino acids (cystine, lysine, etc.). The function, fate, and method of transport of fats, which constitute about a third of our diet, are little understood.

Waxes are widely distributed in plants and animals, serving mainly as protection. The wax coating on leaves and fruits protects against freezing and a too rapid loss of water.

Lipoids may be defined as ether-soluble constituents of tissues without regard to their nature, or, as Leathes says, they are substances that have any of the properties of oil so long as they do not have them all. While biologists have included the sterols (*e.g.*, cholesterol) among the lipoids, and chemists have on occasion used the term to include all the fats and fatlike substances (as lipide is used by Bloor), it is best to restrict "lipoid" to the phospholipides or phosphatides, thus excluding the sterols. Among lipoids are such less well-known fatlike substances as the carbohydrate containing glyco- and galactolipins (cerebrosides) and sulpholipins. As all lipoids can be and are best grouped under various other terms, it is well to consider them there.

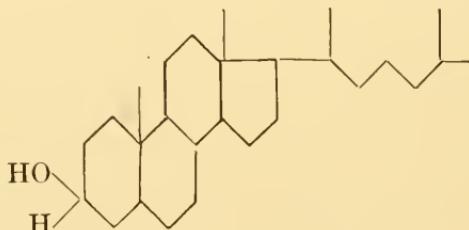
Phosphatides include lecithin, cephalin, and sphingomyelin. The last mentioned is an unsaturated amino alcohol with the probable empirical formula $C_{17}H_{35}NO_2$. It occurs in brain, liver, and egg yolk. Lecithin and cephalin have been known longer. They are always found together and occur in all tissues. Both contain two molecules of fatty acid (*e.g.*, stearic, palmitic, or oleic), one of phosphoric acid, one of glycerol, and one of a base (which is choline in lecithin and amino ethyl alcohol in cephalin).

Lecithin is the most abundant of the phosphatides and is widely distributed in the plant kingdom. It, like other lipoids, is finely dispersed in protoplasm, probably in part as an emulsion.

Sterols are solid alcohols and are distinguished by the hydroxide group. They occur in all organisms (with the possible exception of bacteria), being rather concentrated in the human brain.

Biologically, they may be regarded as of two groups—the plant sterols, or phytosterols, and the animal sterols. Cholesterol is the chief sterol of animals and until recently was thought to be the only one, but others have since been found (*e.g.*, ostreosterol in oysters; agnosterol and lanosterol in wool fat). As cholesterol does not occur in plants and as the several plant sterols are not common in animals, there appears to be a distinction between the plant and animal kingdoms in their sterol content. There is one sterol that is found in both kingdoms, *viz.*, ergosterol; it is essentially a plant sterol but always occurs with cholesterol in animals. Cholesterol may apparently be synthesized by animals, but ergosterol must be obtained by animals from plants.

Cholesterol was one of the earliest known sterols. It consists of three rings of six carbons each, one of five carbons, a side chain of eight carbons, two methyl groups, and one molecule of water, thus:



which yields the formula $C_{27}H_{46}O$. Among the 1,024 possible isomeres of cholesterol, 2 are known with certainty, and several others postulated. (A number of the supposed isomeres of cholesterol are probably distinct sterols, as is true of "iso-cholesterol," now known as lanosterol.)

Ergosterol is second in importance among the sterols so far as our knowledge goes. It appears always to accompany cholesterol. Its formula $C_{28}H_{44}O$ presents an interesting problem, *viz.*, how to fit an extra carbon into the structural formula for cholesterol (as given above). Ergosterol was discovered by the French apothecary Tanret (about 1888). He isolated it from ergot of rye, *Claviceps purpurea*. It is of common occurrence in fungi; its present commercial source, however, is yeast. (Three or more sterols occur in yeast.)

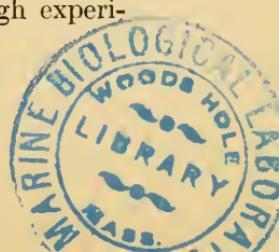
Ergosterol has come into prominence of late because of its antirachitic properties when irradiated with ultraviolet light.

A shift in one double bond of ergosterol produces vitamin D. This is accomplished by irradiation.

Following the discovery of vitamin D by McCollum in 1921 there came a succession of announcements concerning it; Barger of Edinburgh found that ergosterol could be activated by sunlight so as to confer upon it antirachitic properties. Windaus of Göttingen confirmed this and carried the work further. Hess of New York announced that certain foods could be endowed with antirachitic properties. Steenbock of Wisconsin soon followed with work on the activation of foods by ultraviolet rays. Then (1926) Barger showed that ergosterol and not cholesterol is the sterol fraction in which the activity resides and which becomes effective when irradiated. Ergosterol is now believed to be the only sterol that can be endowed with antirachitic properties by irradiation.

When ergosterol is irradiated with ultraviolet light, it is activated, that is to say, converted into a form that is potent from one that has no curative action whatever on rickets. Either sunlight or artificial illumination (if rich in ultraviolet) is successful for radiation. That part of the ultraviolet spectrum lying between 315 and 240 m μ is used. The physical change caused by radiation is assumed to be a reorientation in molecular structure or in electronic arrangement; that is to say, activation consists not in a chemical change such as oxidation but in an isomerization—a rearrangement of bonds (as already stated, probably a shift in one of the double bonds). Only substances containing ergosterol, such as cholesterol, can be activated by radiation. Vitamin D in natural cod-liver oil is believed to be activated ergosterol. When ergosterol is radiated to its maximum amount, it is 250,000 times more potent than cod-liver oil, which has only 1 part in 20,000,000 of the active principle. If radiation is carried too far, the potency drops. The activated state of ergosterol lasts only a few days unless the substance is dissolved in an oil; it is then good for months. In this form, it is sold commercially for use in medicine. C. E. Bills separated an ergosterol mixture into two related sterols, one of which was ergosterol and the other isoergosterol.

There is no trustworthy chemical or physical test for the activated state of ergosterol, the only test for its potency as a cure for rickets being a physiological one (*i.e.*, through experi-



ments on rats). Extraordinarily large doses of vitamin D, e.g., ten thousand or more times the therapeutic dose, result in the deposition of calcium salts in all tissues of the body. This can be carried so far as to mummify a rat.

The final step in the study of irradiated ergosterol has been its synthetic manufacture. This has been accomplished by C. E. Bills, who converted ergosterol into vitamin D by treatment with nitric oxide. The yield was only about 1 per cent. The artificial and natural forms of vitamin D are not identical, though the former has many of the properties of the latter.

Three of the five substantial vitamins are associated with fats—the growth vitamin A, the antirachitic vitamin D, and the reproductive or fertility vitamin E. The first two occur in fish-liver oils, the fat of egg yolk, milk, and green vegetables. Vitamin E is found in butterfat, wheat germ, and lettuce (page 515).

Other biologically important sterols are being determined to such an extent that the fats, in particular the sterols, are assuming great significance—far greater than heretofore realized—as activators and organizers of bodily activities; indeed, they are encroaching upon the proteins as the chief constituents of living matter. In addition to the two sterols (cholesterol and ergosterol) so far discussed in regard to their close association with several vitamins, there are numerous others or derivatives of them which play very important parts in physiological reactions. There is an isomere of ergosterol known as toxisterol which is poisonous. Another is a heart poison. One of the bile acids is a sterol or derivative. The male and the female sex hormones are metabolic products of cholesterol with sterol characteristics (the latter is androsterone). The “organizer” in embryonic development (page 510) is probably a sterol, and the cancer producing hydrocarbon is a sterol derivative.

Other fats, fatlike substances, and fatty acids have important biological functions. Carrel and Baker found the growth-inhibiting effect of blood serum on tissue cultures to be due largely to lipoids. Diabetes appears to be due to an excess of fatty acids.

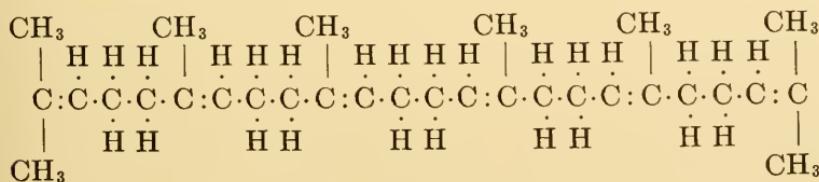
Fatty substances lower surface tension; they will, therefore, aggregate at interfaces. This fact (the principle of Gibbs, page 162) and experimental determinations of electric conductance indicate that the surface layer of protoplasm is rich

in lipoids. Lecithin is rather strongly hydrophilic; it takes up water readily and emulsifies quickly; it may, therefore, function in determining the permeability of cells (page 289). It is also possible that fatlike substances play their part in determining cell permeability not because of the emulsions they form but because of the orientation of their distinctly linear and often polar molecules. (This important problem is discussed in detail on page 256.) That fat molecules may be linear is indi-



FIG. 175.—Protoplasmic processes formed at the surface of unripe Echinoderm eggs in the presence of sperm.

cated by the molecule of squalene (in this case a nonpolar one), discovered by Tsujimoto. It is the chief constituent of the liver oils of elasmobranch fish and has been synthesized by Karrer thus establishing the following formula:



Another likely role of lecithin is its part in the formation of protoplasmic processes, such as surface protrusions, papillae, and small pseudopodia. Whiplike structures, which may be unusual and useless or common and functional, often appear on the surface of protoplasm. Certain of these (Fig. 175) may be identical with the *myelin* forms produced by lecithin when in contact with water, alkalies, acids, etc. These surface outgrowths from lipoids were first described by Virchow and have more recently been carefully studied by Leathes (Fig. 176).

The formation of myelin forms appears to rest upon the hydrophilic qualities of lecithin. The phosphatide molecule becomes hydrated, producing a low interfacial tension between it and

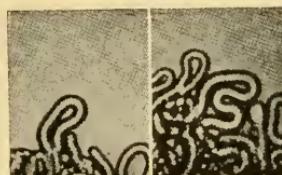


FIG. 176.—Myelin processes formed by lecithin in contact with acid. (From J. B. Leathes.)

water in comparison with the high value ordinarily existing between a true fat and water. The number of molecules of lecithin in contact with water at the surface tends to increase because of low interfacial tension, and the surface of the lecithin advances into the water; myelin forms result. It is likely that many superficial protoplasmic processes arise in the same way. F. Weber has described myelin forms from the chloroplasts of Spirogyra when put into a soap (sodium oleate) solution. Protoplasmic processes (similar to those in Fig. 173) were first described by Fol as coming from the surface of ripe echinoderm eggs. These may serve to attract or guide sperm. Bacteria and many types of one-celled organisms develop delicate protoplasmic protrusions (*e.g.*, cilia) which are functional; these processes may, however, be of protoplasm and not of lipoid alone.

CHAPTER XXV

PROTEINS

On several occasions, reference has been made to the proteins as the most important constituents of protoplasm. While it is impossible to single out any one necessary substance as the most important in a system, yet, as Pauli has said, the proteins, more than any other group of substances, display those properties which combined we call life. Obviously, then, a study of them must yield very important deductions in the physics and chemistry of protoplasm.

Emil Fischer regarded the proteins as taking part, in one way or another, in all physiological processes in the living organism.

The early study of proteins was by classical chemical methods. Only recently has the colloid chemist carried the study of them into the world of physics. Among the first to do this were the Scandinavian S. P. L. Sørensen and the Austrian physician Wolfgang Pauli. It is our task to consider the physical properties—in particular, the structure—of the proteins. It was an elucidation of their structure that Emil Fischer advocated in pointing out the significance of the study of proteins in biological chemistry. But before the physical aspects of protein behavior can be satisfactorily considered, some knowledge of their chemical constitution must be had. For this, we turn to the organic chemists.

Classification.—A classified list, with examples, will convey an idea of the kind of substances that proteins are better than will a definition. The English classification has the advantage of simplicity; with slight modifications, it is as follows:

- I. Simple proteins—These are naturally occurring proteins which are hydrolyzed only by enzymes or acids into α -amino acids or their derivatives
 - A. Protamines—salmin from salmon sperm
 - B. Histones—globin from hemoglobin
 - C. Globulins—ovoglobulin from egg yolk

- D. Albumins—white of egg
 - E. Glutelins—glutenin from wheat
 - F. Prolamins—gliadin from wheat
 - G. Scleroproteins—keratin from hair, gelatin from bones
 - H. Phosphoproteins—casein from milk
- II. Conjugated proteins—These are compounds of simple proteins with some other nonprotein group, the union with the nonprotein molecule being otherwise than as a salt
- A. Chromoproteins (hemoglobins)—proteins of wool and hair; the nonprotein group is a pigment
 - B. Nucleoproteins—proteins of cell nuclei; the nonprotein group is nucleic acid
 - C. Glucoproteins—mucin from the salivary glands; the nonprotein group is a carbohydrate radical
- III. Hydrolyzed proteins (protein derivatives)—This is an artificial group and includes the various decomposition products of the naturally occurring proteins.
- A. Metaproteins—acid and alkali albuminate
 - B. Proteoses—partial hydrolytic decomposition products of proteins (precipitated by ammonium sulphate)
 - C. Peptones—partial hydrolytic decomposition products of proteins (not precipitated by ammonium sulphate)
 - D. Polypeptides—compounds of two or more amino acids

The phosphoproteins have, as the name indicates, a nonprotein group containing phosphorus, yet in the English classification they are put under simple proteins.

Atomic Constituents.—All proteins contain carbon, hydrogen, oxygen, and nitrogen; and all, except the protamines, histones, and their derivatives, contain sulphur. These elements may occur in proteins in the following elementary proportions: carbon, 51.55 per cent; hydrogen, 7 per cent; oxygen, 20 to 30 per cent; nitrogen, 15.17 per cent; sulphur, 0.4 to 2.5 per cent, from which a formula such as $C_{726}H_{1174}N_{194}S_3O_{214}$ for globin, the basis of hemoglobin, can be calculated. Iron and magnesium are other common protein constituents. The phosphoproteins and nucleoproteins contain phosphorus. The accessory elements sulphur, iron, magnesium, phosphorus, etc., do not always occur as a direct part of the protein molecule but are combined with atomic groups to form definite chemical substances which are more or less firmly bound to the purely protein constituents of the larger complex which we call a protein molecule. Phosphorus in the phosphoproteins is probably combined directly with one of the constituents of the protein molecule, but in the nucleo-

proteins it is combined with a purine base or carbohydrate, forming nucleic acid.

Amino Acids.—When proteins are broken down into their simple component parts by the process of hydrolysis through the intermediary of heat, enzymes, or other reagents, there result, first, the polypeptides (and other protein derivatives mentioned in group III) and, finally, substances known as the *amino acids*. These latter are the "building stones," or structural units, of the proteins. So far, 31 amino acids are known. The first and simplest of them is glycine, or glycocoll (alpha amino acetic acid). It was the second amino acid to be discovered (by Braconnot in 1820), having been preceded, historically, by leucine (discovered by Proust two years earlier).

The basis for recognizing an amino acid is now generally conceded to be its isolation by some worker other than its discoverer, and the determination of its constitution by synthesis. On this basis, twenty-one amino acids are recognized. They are (in order of their discovery):

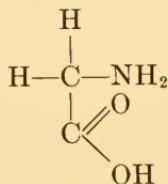
Leucine	3.5 di-iodo-tyrosine
Glycine	Cystine
Tyrosine	Tryptophane
Serine	Proline
Aspartic acid	Cysteine
Glutamic acid	Hydroxyproline
Alanine	Isoleucine
Phenylalanine	Valine
Lysine	β -alanine
Arginine	Thyroxine
Histidine	

The total number reported is now nearly double that given above, some of which may be authentic but not yet have met the test of recognition. Their technical names are of interest only to the specialist; for example, leucine is β -isopropyl- α -amino-propionic acid. The amino acids are listed above in the order of their discovery, which is also almost the order of complexity. Glycine is the simplest. The list is not yet complete, as in no instance does the total number of amino acids isolated come to much more than 70 per cent of the weight of the original protein. The majority of the amino acids are commonly found among the hydrolytic products of the proteins, although their relative

proportions vary considerably. Casein yields some 20 amino acids.

Proteins may be defined as substances that on hydrolysis yield amino acids. The intermediate decomposition products, such as the polypeptides, are not proteins in the strict sense but rather are protein derivatives which in their turn yield amino acids.

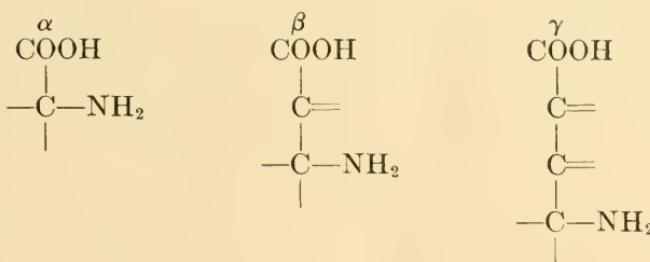
An amino acid may be defined as an acid in which one or more of the hydrogen atoms, other than the carboxylic hydrogen, is replaced by the amino radical NH_2 . For example, by replacing one of the hydrogen atoms attached to the carbon atom in acetic acid, CH_3COOH , with the amino radical, NH_2 , glycine (glycocol), $\text{CH}_2\text{NH}_2\text{COOH}$, the simplest of the amino acids, results. Theoretically, it should be possible to replace two or all three of the hydrogen atoms in the CH_3 group of acetic acid by amino radicals and get diamino and triamino acetic acid, but these two compounds are unknown. The orientation of the atoms in glycine is presumed to be as follows:



While such structural formulas are the nearest possible approach at present to atomic orientation in protein derivatives, the amino group may not be present in the protein molecule as such but possibly in the form of NH groups.

The next homologue in the fatty-acid series after acetic acid is propionic acid, $\text{CH}_3\text{CH}_2\text{COOH}$. It yields two monoamino acids (*i.e.*, acids with one amino radical each)—alpha and beta aminopropionic acid, $\text{CH}_3\text{CHNH}_2\text{COOH}$ and $\text{CH}_2\text{NH}_2\text{CH}_2\text{COOH}$, respectively. These two amino acids are distinguished by the position of the amino radical, NH_2 , which is attached to the alpha carbon atom—the carbon adjacent to the carboxyl group—in the first case, and to the beta carbon atom—next but one from the carboxyl—in the second case.

The relative positions of the amino and carboxyl radicals determine the type of amino acid. These may be indicated as follows:



Thus, we see that the alpha, beta, and gamma types of amino acids are determined by the distance that the NH_2 radical is separated from the COOH group. If there are two amino radicals at different distances from the carboxyl group, the amino acid belongs to both of the two types; thus, valeric acid, $\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$, when converted into diaminovaleric acid, $\text{CH}_3\cdot\text{CH}(\text{NH}_2)\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$, becomes an amino acid of the alpha and gamma type. An amino acid to be strictly and solely of one type can contain only one amino group. Glycine is such a pure alpha type. The alpha type of amino acid is by far the most common and important. Only rarely does the beta type occur in nature. All of the acids that are products of the hydrolysis of proteins are alpha amino acids.

A definite number of amino acids, in specific arrangement, build up each protein. The table shown on page 474 is the constitution of egg albumin, the names, proportions, and chemical constitution of the eight different amino acids being given.

There are eight kinds of amino acids in albumin, but many more than eight amino acid chains or molecules, for of each kind there are from few to many units. A conservative estimate of three molecules for the least abundant member—cystine—would give a total of over 250 amino-acid chains in one molecule of albumin.

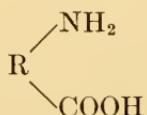
Synthesis.—Hydrolysis of the proteins causes them to break down into their component parts. It should, therefore, be possible to reverse the process and build up proteins by uniting amino acids with the elimination of water. This is how Fischer accomplished the synthesis of simple protein derivatives. The first stage in such a condensation process results in the formation of a dipeptide (glycyl-glycine). A third molecule of the same amino acid, or a different one added by condensation, yields a *tripeptide*. The most complex *polypeptide* yet produced is an

octadecapeptide the molecule of which contains 15 glycyl ($-\text{NH}\cdot\text{CH}_2\cdot\text{CO}-$) and 3 leucyl ($-\text{NH}\cdot\text{CH}(\text{C}_4\text{H}_9)\cdot\text{CO}-$) groups. The molecular weight of this substance is 1,213, and its properties are much like those of the natural proteins.

EGG ALBUMIN

Amino acid	Proportion	Chemical constitution
1. Alanine.....	2.1	$\text{CH}_3\cdot\text{CH}\cdot\text{NH}_2\cdot\text{COOH}$
2. Leucine.....	6.1	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}\cdot\text{CH}_2\cdot\text{CH}\cdot\text{NH}_2\cdot\text{COOH} \end{array}$
3. Phenylalanine	4.4	$\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CH}\cdot\text{NH}_2\cdot\text{COOH}$
4. Tyrosine.....	1.1	$\text{OH}\cdot\text{C}_6\text{H}_4\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$
5. Cystine.....	0.3	$\text{COOH}\cdot\text{CH}(\text{NH}_2)\cdot\text{CH}_2\text{S}=\text{S}\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\text{COOH}$
6. Proline.....	2.3	$\begin{array}{c} \text{CH}_2-\text{CH}_2 \\ \qquad \\ \text{CH}_2 \qquad \text{CH}\cdot\text{COOH} \\ \backslash \qquad / \\ \text{NH} \end{array}$
7. Aspartic acid.	1.5	$\text{COOH}\cdot\text{CH}_2\cdot\text{CHNH}_2\cdot\text{COOH}$
8. Glutamic acid	8.0	$\text{COOH}\cdot\text{CHNH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$

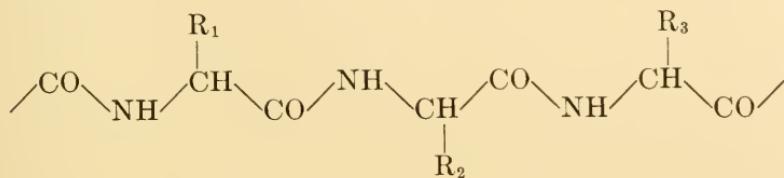
Protein Structure.—The fact that the proteins can be broken down into their component parts and the even greater achievement of building them up by combining these parts have led to some knowledge of their structure. Where this knowledge is lacking, and in all cases as a matter of convenience, that part of the protein molecule about which we know little is indicated by the letter *R*; attached to it are the two known radicals NH_2 and COOH , which form a part—and so important a part—of every protein molecule. The structural formula becomes simply



However, much more than this has been attempted in the way of an interpretation of protein structure. The protein molecule and its derivatives are generally recognized as possessing the

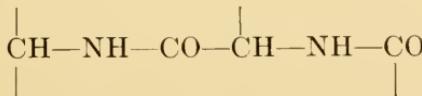
form of a chain, or thread, which may possibly in certain cases be regarded as a tenuous, crystalline fiber. This interpretation is based in part on the fact that the proteins are built of amino acids the molecules of which are linear. The synthesis of polypeptides led to four possible structural formulas for glycyl-glycine; of these, Fischer accepted the following linear orientation: $\text{NH}_2\text{—CH}_2\text{—CO—NH—CH}_2\text{—COOH}$.

Carrying out the idea of a linear structure for the amino acids, polypeptides, and proteins, we obtain the following chain for a protein molecule:



The —NHCO— union, which occurs recurrently in the above structural formula, is known as the *peptide linkage* and is regarded as the one most typical of proteins, though not the only one. By means of this and other links, amino acids combine to form polypeptides; polypeptides (with other substances), to form simple proteins; and simple proteins, to form complex proteins.

While the linear unit is widely recognized as characteristic of proteins, it may unite with other like units to form other figures such as closed rings, or it may itself be a ring. The latter case is illustrated by the "peptine ring":



Larger and more intricate cyclic compounds, or ring structures, are presumed to exist, but the evidence is not conclusive.

The ring type of protein receives support from the centrifuge experiments of Svedberg, who, from determinations of molecular weight, estimates the diameters of protein molecules, thus suggesting that they are spherical. It may be that the protein thread is coiled into a ball. The consensus of opinion, however, is that the protein molecule is linear. A very possible form which has been suggested is a helix. The helical spiral, while

composed of a linear fiber and itself an elongated structure, gives substantial thickness to the molecule as a whole.

We have to deal not only with the question of the external shape of a protein molecule but also with the difficult one of internal arrangement of parts. Any change in the orientation of the amino acids will mean a different protein. The apparent hopelessness of the situation is graphically given by the English chemist J. B. Leathes. He takes the "very simple case" of a protein with only 50 amino links. If 1 link recurs ten times, 4 recur four times, and 10 recur twice, then the number of possible permutations will be, $\frac{50}{10} \times (4)^4 \times (2)^{10}$. In such a protein of 50 links, of which only 19 are different, the number of possible arrangements of its parts will be 10^{48} . Light takes 300,000 years to travel the length of the Milky Way. This distance, expressed in angstrom units, of which 100,000,000 equal a centimeter, will be less than 10^{32} . It is thus clear how great the variations in disposition of the parts of a protein molecule may be and how far we are from being able to map out such a structure.

Molecular Weight.—Among the many distinguishing properties of the proteins we shall select but a few, the essentially colloidal ones, in which we, as biologists, are primarily interested, though there are others such as optical properties (proteins are laevorotatory) which are equally significant.

That proteins are colloidal systems there can be no doubt, but until their structure and behavior are better known it cannot be said with certainty whether they are colloidal because of their huge molecules alone or because of a larger structural unit—the micelle. Structure is significant, but we shall do well to remember that we ask of colloidal systems not what they are but what they do. The fact that proteins diffuse slowly, exert little osmotic pressure, exhibit a Tyndall cone, and form jellies is sufficient evidence that they are colloidal quite aside from the nature of their dispersed particles. Protein molecules are sufficiently large to account for colloidal behavior without the need of postulating molecular aggregates—the micelles—even though these may be present.

The molecular weight of hemoglobin has been variously given, from a minimum of 16,000 to a maximum of 68,000. The lower estimate was based on the assumption that the molecule

($C_{758}H_{1203}O_{195}N_{218}FeS_3$) contains but one atom of iron. This and other low estimates are now regarded as inexact, the value 68,000 being the at present accepted weight of hemoglobin. Three investigators, using quite different methods, have found the molecular weight of hemoglobin to be 68,000, Svedberg using the ultracentrifuge; Adair, on the basis of osmotic pressure; and Northrop, from diffusion rate. The molecular weight of egg albumin was first (by Sørensen) put at 14,000, on the basis of freezing-point determinations of osmotic value. But again the first estimate was too low. The value now generally accepted for egg albumin is 34,500, determined by Svedberg. It is probable that few if any proteins have a molecular weight of less than 10,000 (possibly 34,500), as compared with 18 for water and 342 for cane sugar.

The method of Svedberg for determining the molecular weight of substances with heavy molecules is one of the most recent and most ingenious. By means of a centrifuge, Svedberg has been able to cause sedimentation in protein solutions—in itself a startling fact, as heretofore it has not been supposed that molecules would settle out under centrifugal force. Two factors are involved—equilibrium of sedimentation (gravity) and velocity of sedimentation—from either of which the molecular weight can be calculated. The maximum force attained is two hundred thousand times that of gravity. Hemoglobin, with a molecular weight of 68,000, requires a centrifugal force of about fifty thousand times gravity to clear the top layer. Egg albumin, with a weight of 34,500, is one of the lightest of protein molecules. Svedberg believes that proteins of higher molecular weights are exact multiples of this value. Maximum values are reached in the naturally occurring pigments of plants, such as phycocyanin and phycoerythrin from algae. The molecular weight of both of these proteins is 208,000. The highest molecular weight determined by Svedberg is that of the blood pigment hemocyanin from the snail. It has the enormous value of 5,000,000. Whether or not the values obtained are of molecules or of molecular aggregates (colloidal particles) cannot be said with certainty, nor is 34,000 necessarily a minimum value. Svedberg speaks of low-molecular “proteins” occurring in the living organism and brought into the shape of a “real” protein of higher weight by a “purification” process.

The following molecular weights of proteins are fairly exact multiples of the first: egg albumin, 34,500; hemoglobin, 68,100; serum globulin, 103,600; phycoerythrin, 208,000.

The problem of determining the molecular weights of proteins is difficult not only because of the large size of the molecules and our comparative ignorance of their constitution but also because the molecular weight is apparently not constant, differing under different conditions. Svedberg believes that this is true for acid and alkaline solutions and that the molecular weight is lowest in solutions of high alkalinity. Burk finds hemoglobin in urea to have one-half the molecular weight that it has in water, while albumin is the same in both solutions. Apparently, depolymerization (a breaking down of the molecule) of hemoglobin takes place in urea. Possibly, the situation is as in the case of cellulose, where the molecule is a chain of anhydrous glucose links the number of which may vary. The chain, and therefore the molecule, is of indefinite length and weight. This concept, fairly well established in the case of cellulose, is contrary to classical laws, which demand constancy in size, weight, and constitution of molecules; but classical laws have often had to give way to newer ideas.

The situation in regard to the relationship between pH and molecular weight of proteins appears to be that only within a definite pH range, which is characteristic for every protein, is there a well-defined molecular weight. The isoelectric point is always within this range. Outside the range (acid or alkaline), the molecules split up and give other "molecular-weight" values.

The suggestion of Svedberg that the molecular weights of proteins are all multiples of 34,500 and that if the higher weights are regarded merely as polymerized forms of the lower unit, then "all proteins have the same molecular weight" is not as yet generally accepted, even though his measurements support the idea. Determinations of molecular weights from chemical data (as opposed to Svedberg's physical centrifuge method) give the values¹ shown in the table on page 479.

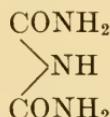
Crystalline Protein.—One of Graham's criteria of the colloidal state was the absence of a crystalline condition; this distinction has proved to be erroneous, for many proteins can be crystallized

¹ From Burk.

without difficulty. Oxyhemoglobin, egg and serum albumin, certain vegetable proteins, and others are obtainable in the form of well-defined crystals.

Protein	Minimum molecular weight from chemical data	Molecular weight in aqueous solution	Molecular weight in urea solution (from osmotic pressure)
Casein.....	12,800	98,000	33,600
Hemoglobin.....	16,660	67,000	34,300
Egg albumin.....	33,800	34,000	36,000
Serum albumin.....	78,000	75,000	73,000

Color Reactions.—Color reactions were formerly thought to be characteristic of definite kinds of proteins, but we now know that most of these reactions are due to special amino-acid groups. The *biuret* reaction gives a red or violet color when an excess of caustic soda and a trace of copper sulphate are added to a protein solution. This reaction is given by all proteins, by proteoses, peptones, and nearly all of the synthetic polypeptides, but the test fails with individual amino acids. The reaction receives its name from biuret (*bi* + *urea*), $C_2H_5N_3O_2$, a substance formed by exposing urea to high temperatures:



The formula of biuret suggests that the color reaction is given by substances having two amino groups (NH_2) in their molecule.

Strong sulphuric acid added to a protein solution containing alcoholic alpha-naphthol produces a violet (or red) color. The reaction is given by all proteins that contain carbohydrate complexes and depends upon the production of furfural.

Coagulation.—*Coagulation*, in the broadest meaning of the term, includes a variety of phenomena some of which may be identical, some very similar, and some quite distinct, yet all are called by this name. Coagulation takes place when blood comes into contact with air, when rennin or bacteria are added to milk, or when proteins are heated. These are true coagulation

phenomena. But there are other processes which are either actually or superficially very similar to coagulation. One of these is *precipitation*. If hydrochloric acid is added to a solution of silver nitrate, silver chloride is precipitated. When the muddy waters of the Mississippi River meet the salt water of the sea, the mud is precipitated. These two events appear to be identical, but actually they are due to quite distinct happenings. The precipitation of the silver salt is due to a chemical exchange of ions. The precipitation of the suspended clay particles in the river is due to surface phenomena involving no interchange in a chemical sense. Only the latter is true coagulation.

Other processes which are similar to coagulation, in that they involve the production of solid from dispersed matter, are *gelation*, *gelatinization (setting)*, *coalescence*, *flocculation*, *agglutination*, and *salting out*.

Gelation is the change that a colloidal suspension undergoes in forming a reversible or irreversible gel. It will be recalled (page 138) that we decided to use the term "gel" to indicate both jellies (reversible, elastic, nonporous gels of the type of gelatin) and coagula (irreversible, relatively inelastic, porous gels of the type of silica gel). Thus does gelation become the solidification of the sols of both jellies and coagula. *Gelatinization* is the setting, or gelation, of a reversible gel or jelly. A solution of gelatin gelatinizes into a readily reversible jelly, while one of silicic acid coagulates into an irreversible, inseparable mass of silica. Both gelate or set. *Coagulation* may be defined as the irreversible aggregation of particles into an inseparable mass. But such a definition eliminates those processes where irreversible but separate masses are formed and also all reversible forms of aggregation. (By reversibility is usually meant a return to the original state, liquid or solid, by a reversal of the *same* process that produced the new, solid or liquid, state; thus, gelatin is reversed by merely reversing the process of heating or cooling which caused solation, or gelation, but a boiled egg cannot be so reversed.)

To restrict coagulation to irreversible gelation is arbitrary though often done. Coagulated sols of iron oxide, vanadium pentoxide, and arsenous sulphide may be reversed when weak electrolytes are used and not too long a time allowed for the coagulation, yet the process is distinctly coagulation. We may

then distinguish between irreversible and reversible coagulation. A sharp distinction is to be made between the coagulation of metal sols by added electrolytes and that of proteins by heat.

Coalescence is the fusing of (usually) liquid droplets to form a continuous mass of the liquid substance. It takes place in the breaking of emulsions, as in the churning of cream to form butter.

Flocculation is a form of coagulation involving the clumping together of ultramicroscopic particles into outwardly visible and usually slowly falling discrete particles. The word distinguishes only a superficial appearance.

Agglutination is a term applied to the aggregation of living cells such as bacteria, which come together when a salt or immune serum is added to them and form a reversible mass. As the bacteria can be shaken apart and redispersed, the clumping together is a form of reversible coagulation.

Salting out differs from certain other forms of coagulation in that it involves dehydration rather than decharging. It takes place only at high salt concentrations. (The term is used in soap manufacture.)

We may, then, distinguish at least four main types of phenomena which cause molecules or particles in suspension to come together and fall, or form a solid mass, *viz.*, precipitation (in the strict chemical sense), the coagulation of metal sols (by electrolytes), the coagulation of proteins (by heat, salts, enzymes, etc.), and the gelatinization (setting) of jellies.

The Mechanism of Coagulation.—The coagulation of a positively charged colloidal suspension is accomplished by the addition of a negatively charged suspension. That the charges, by neutralizing each other, are actually responsible for the precipitation can be shown by adding a colloidal suspension of the same charge as to sign and by contrasting the effects of electrolytes with those of nonelectrolytes. While there can be no doubt that certain precipitation and coagulation phenomena are electrical in nature, in that they involve a reduction in surface charge, there are some interesting cases where reduction in charge is not the initial but the final step in the process. Thus, suspensions of *like* charge are not indifferent to each other and may lower stability when combined. This is true of sulphur and arsenous sulphide solutions—both negative—and of sulphur and silver solutions—also both negative. Radiation lowers the stability

of certain solutions; so also does an alteration in the dielectric constant of the medium through the addition of alcohol, acetone, etc. Chemical change in the environment of the particle, due to reactions between the substance added and the substance adsorbed on the surface of the particle, may be responsible. Silver ions in the stabilizing envelope of silver particles will react with added sulphides; this would strip the metallic colloidal particles of their charge. A chemical reaction is thus the primary cause; reduction in charge, the ultimate one.

The precipitation of smoke and of fog is again a phenomenon the mechanism of which appears to differ from coagulation.

Dehydration is a cause of coagulation which seemingly functions wholly apart from electric charge. Proteins may be kept in suspension by a water envelope (page 147). Kruyt does not believe that it alone is sufficient; however, if it is removed, coagulation may result.

The use of salts for precipitating or salting out proteins and other organic compounds is one of the best means of purification. All proteins are coagulated on complete saturation of the solution with ammonium sulphate. Other substances employed as coagulants are sodium chloride, sodium sulphate, zinc sulphate, magnesium sulphate, and organic liquids such as alcohol, ether, and acetone. Proteins differ widely in the relative ease with which they are coagulated by salts. Ease of coagulation of proteins appears to be correlated with molecular weight, as shown by the behavior of globulin and albumin from blood serum; the former of high molecular weight is more easily precipitated than the latter of lower weight. (Any correlation between coagulation and molecular weight is probably due to other factors of which molecular weight is only an index.)

The relative effectiveness of salts as precipitants brings us again to the question of Hardy's valency rule and the Hofmeister series. When colloidal suspensions of metals or their salts are coagulated by ions, an almost strict valence effect is obtained (page 172); but in proteins, where hydration may play a greater role than charge in determining stability, the ions become arranged in a typical Hofmeister, or lyotropic, series, as Hofmeister showed in the original series (page 445).

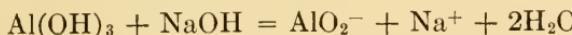
The discussion of coagulation is continued in relation to living systems (page 492).

Amphoteric Properties.—Proteins and their structural units the amino acids have the power to combine with both acids and bases so as to form salts; that is to say, a protein such as gelatin will react with a base such as sodium hydroxide or with an acid such as hydrochloric to form a salt—in the former case, sodium gelatinate; and in the latter, gelatin chloride. Because they possess both acid and basic characters, proteins are said to be *amphoteric*. All substances are amphoteric if they possess groups that can take on or give up the hydrogen ion. Bredig was a pioneer investigator of amphoteric electrolytes, and he defined them as substances that play the part of an acid toward a base or the part of a base toward an acid; in other words, they are substances that split off or combine with H^+ or OH^- ions. Mann points out that, in this sense, water is an amphoteric electrolyte because its hydrogen atom and its hydroxyl radical may be converted into the chemically active H^+ and OH^- ions whenever water comes into contact with certain salts (see also page 373).

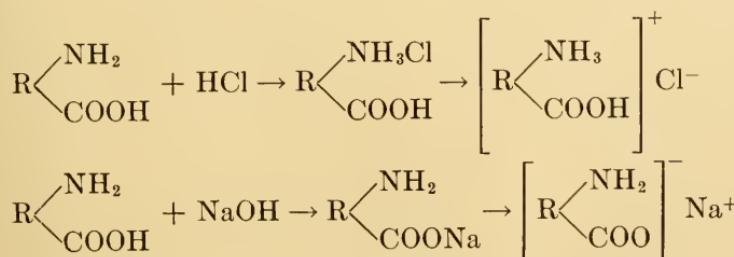
The hydroxyl derivatives of many elements from the middle of the periodic table, such as aluminum, chromium, zinc, lead, tin, manganese, arsenic, and antimony, all behave like weak bases or acids and are, therefore, examples of amphoteric electrolytes among inorganic compounds. It is not the metals but their hydroxides that are amphoteric; thus:



and

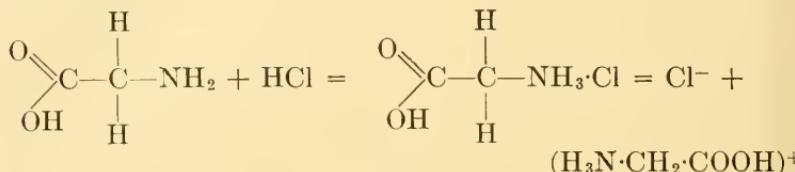


If we represent the remainder of the protein molecule by R, to which are attached the amino and carboxyl radicals, then we can illustrate the amphoteric properties of proteins in the presence of acids or bases as follows:

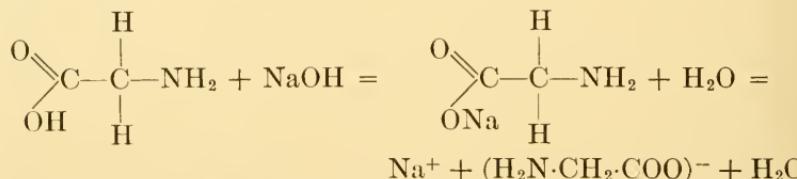


A protein is thus positive in an acid medium and negative in an alkaline one.

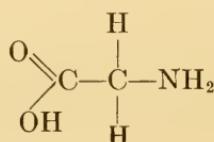
The foregoing story can be retold to advantage by using a simple protein derivative. The simplest amino acid is glycine; in the presence of an acid (hydrochloric), it forms glycine hydrochloride, as follows:



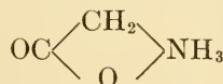
In the presence of a base (sodium hydroxide), glycine forms sodium glycinate and water:



As the acid tendencies of proteins are contributed by the carboxyl radical (COOH) and the basic ones by the amino radical (NH_2), the individual amino acids themselves should exhibit amphoteric properties *within* the protein molecule, in that they join, one to another, by linking the amino group of one amino chain with the carboxyl group of another; thus, the diamino molecule of glycyl-glycine is $\text{H}_2\text{N}\cdot\text{CH}_2\cdot\text{CO}-\text{NH}\cdot\text{CH}_2\cdot\text{COOH}$, and the triamino molecule of diglycyl-glycine is $\text{H}_2\text{N}\cdot\text{CH}_2\cdot\text{CO}-\text{NH}\cdot\text{CH}_2\cdot\text{CO}-\text{NH}\cdot\text{CH}_2\cdot\text{COOH}$. Both are formed by linkage of the carboxyl radical of one amino acid with the amino radical of another amino acid of the same kind, a molecule of water being split off. In this way, an amphoteric molecule may show a tendency toward internal salt formation; that is to say, the acid and the basic radicals may mutually satisfy each other. The open chain would then be converted into a ring, and chemically active glycine



would become chemically inactive glycine:



These older views are still possible even though augmented by newer ideas. Bjerrum and others regard glycine (99 per cent of it) as existing as a *Zwitter* or double ion (the German term *Zwitterion* is generally used for these double ions). Such an ion is cation and anion in one, *i.e.*, possesses both a positive and a negative charge, and may be written $^+\text{NH}_3-\text{R}-\text{COO}^-$, which for glycine becomes, $^+\text{NH}_3\cdot\text{CH}_2\cdot\text{COO}^-$.

Electric Charge.—While organic colloidal particles have been regarded as mammoth ions (page 371), and they may be so in certain aspects of their behavior, there is a fundamental difference between a typical metal colloidal particle and an ion; the electric charge of the latter is due to the loss or gain of an electron, while that of the former is due to the adsorption of ions on to the surface of the particle. Proteins possess electric charges which they owe apparently to both ionic and colloidal properties.

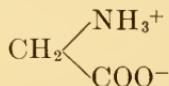
While we are, for the moment, interested in the electric charge on proteins, it must be remembered that the stability (and solubility, etc.) of proteins rests usually in part, perhaps at times solely, upon hydration. Pauli first suggested that hydration is a factor in protein stability. Kruyt and deJong extended the idea to include other substances (agar, etc.) (page 147). Protein particles in aqueous suspension when decharged by electrolytes or dehydrated by alcohol may remain in suspension, but they fall when both are added.

Let us first consider proteins as ions. When in the presence of acids or bases, proteins form salts which ionize. They are, therefore, electrolytes, with acidic or basic properties, and for this reason are known as *ampholytes*, thus indicating both their amphoteric and their ionization properties when in solution. Ions migrate in an electric field. Proteins should, therefore, show cataphoretic flow, and this they do (page 375). The sign of the charge and therefore the direction of migration will depend upon the degree of acidity of the solution. As we have seen, a protein salt, *e.g.*, gelatin chloride or sodium gelatinate, ionizes and in such a way that when in alkaline solution, the protein (gelatin) ion is

negative ($\text{Na}^+ + \text{G}^-$); and when in acid solution, it is positive ($\text{G}^+ + \text{Cl}^-$); consequently, in an electric field, the positive protein ion will travel to the cathode, and the negative one to the anode. Midway between the state of complete combination with acid and complete combination with base will be a point where no migration takes place. This is the isoelectric point. At this point, there is no (or a minimum) migration, minimum combination of the protein with acid or with base, and minimum solubility. The isoelectric point may be in an acid, a neutral, or an alkaline region, depending on the relative number and strength of the acidic and basic groups. As the protein ion is either positive or negative depending on the pH of the solution, it is evident that to state the cationic or anionic nature of a protein salt is meaningless unless the pH of the solution is given. This applies not only to proteins but to living cells which may be coated with protein.

So much for protein salts which ionize into a protein anion in an alkaline solution and into a protein cation in an acid solution. With pure or electrolyte-free protein, the story is slightly different. We still have to do with ionization, not of a protein salt but of amino and carboxyl groups within the protein molecule. Albumin, after weeks of electrodialysis, is almost, if not wholly, free of acid and base, yet the albumin is still slightly negatively charged and exhibits feeble cataphoretic properties.

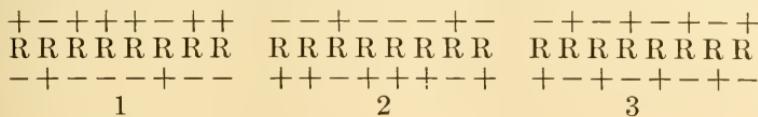
An important concept, expressed by the term *Zwitterion* (literally, hermaphroditic), has been introduced into protein chemistry; it signifies that ionized amino acids are compound ions; thus: $^+\text{NH}_3-\text{R}-\text{COO}^-$, which for glycine, as we have seen, becomes



Amino acids as *Zwitterionen*, or double ions, acquire and therefore give to proteins properties peculiar to themselves. Among these is the possibility of precipitation with either the cation or the anion of a salt. Our present interest in double ions is their orientation within the protein molecule.

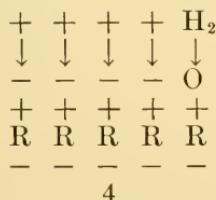
If the numerous NH_2 and COOH groups within a protein molecule are ionized, then whatever the configuration of the protein molecule as a whole may be, some of the ionized NH_3^+ and COO^- radicals will be exposed at the surface, and the excess in number of

the one or other kind will determine the sign of the charge of the electrolyte-free protein molecule. We may imagine three possible surface distributions of the NH_3^+ and COO^- groups, as follows (the upper layer representing the outer surface of the molecule):



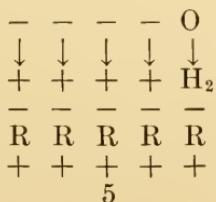
If the orientation is as in case 1, then the protein molecule is predominantly positive; if as in case 2, then it is predominantly negative; and if as in case 3, it is neutral. The negative charge of a pure electrolyte-free protein is due to an excess of the exposed COO^- groups over the NH_3^+ groups.

The charged surface of a protein molecule will attract and hold polar molecules such as those of water. If the orientation of the Zwitterions is as in case 1 above, then the water dipoles will be held by their oxygen ends, thus:



Pauli has illustrated this for colloidal particles with a positive surface charge (Fig. 177).

If the ionic orientation is as in case 2, the water dipoles will be grouped with their positive hydrogen atoms toward the surface of the molecule, thus:



If the orientation is as in case 3, both types of arrangement of the water molecules will exist. Hydration will be more pronounced in case 4 than in case 5, because the negative oxygen end of the water dipole clings more firmly than does the hydrogen end.

The oxygen atom of water, with its double charge concentrated in one atom, is electrically stronger than the two combined hydrogen atoms, with their two positive charges distributed in two atoms (Fig. 138). This fact explains why cations, such as Na^+ (Fig. 177A), or proteins with positively charged surfaces are generally more strongly hydrated than are anions or negatively charged proteins.

A return to experimental facts will add conviction to the essential points, in case hypotheses tend to obscure them. Albumin from blood serum, after the removal of electrolytes by dialysis, moves slowly in an electrical field on prolonged applica-

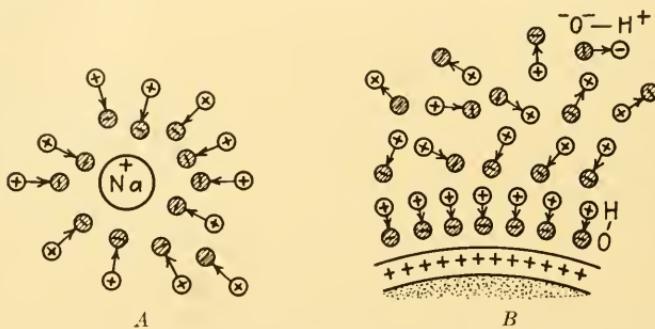


FIG. 177.—A. A hydrated cation. B. A hydrated positive collodion particle.
(From W. Pauli.)

tion. The direction is to the anode, owing to a slight initial negative charge of the protein. Pauli has shown that pure horse serum, dialyzed for seven weeks, migrates to the anode after twenty-four hours of electrophoresis under 250 volts and 2×10^{-5} amp. The addition of alkali or alkaline salt causes a pronounced wandering to the positive pole, while acid or acid salts promote a movement toward the negative pole. Most of the natural proteins (albumin, globulin, glutin, casein, hemoglobin) show a stronger acid than basic character. It frequently happens that at the isoelectric point there is movement in both directions over a short range of hydrogen-ion concentration.

The Isoelectric Point.—Reference to the *isoelectric point* has been made (page 375). It is the point of zero potential on colloidal particles and therefore the point of no migration in an electrical field. It was discovered by William B. Hardy in his work on proteins. Its discovery represents, in a sense, the beginning of the physicochemistry of proteins. Hardy, in 1899,

found that the white of an egg diluted with distilled water, filtered, and boiled migrates in an electric field—in one direction when the solution is acid and in the opposite direction when alkaline. Obviously, when the solution is neutral, there should be no migration. This is the isoelectric point. Neutrality here means electrical and not acid-alkaline neutrality, though the isoelectric point is most often expressed in terms of pH. The isoelectric point is the point of zero potential (or nearly so) of a colloidal particle, and it is usually not at acid-alkaline neutrality, *i.e.*, at pH 7. For proteins, it is apparently always, certainly most often, on the acid side; in other systems, it may be on the alkaline side (rarely, there are two isoelectric points, one acid and one alkaline). The isoelectric point of gelatin is at pH 4.7.

Proteins as Colloidal and Crystalloidal Systems.—There can be no doubt as to the colloidal nature of proteins; their capacity to form gels, to swell in water, and to exhibit the Tyndall phenomenon is typical colloidal. But it may be that under certain conditions they lack one of the most characteristic properties of colloids, *viz.*, the micelle. While this lack, if it exists, may from one point of view be of no great consequence—for the protein molecule is itself sufficiently large to account for colloidal properties—yet there is another side to the question. If proteins are molecularly dispersed when in solution, they should behave like a salt, as we have assumed that they do. If they are colloidally dispersed, they should show those colloidal properties characteristic of surfaces, such as adsorption. Let us consider a simple case. If hydrogen ions disappear from a solution of hydrochloric acid in which gelatin is immersed, then these ions may either have united in stoichiometrical proportion with the gelatin to form a salt (gelatin chloride), all according to the laws of valence, or the hydrogen may have been selectively adsorbed by the gelatin particles according to a colloidal law, such as Freundlich's adsorption isotherm, which does not follow the law of valence. The assumption that proteins form salts with acids and bases is too well supported by experimental facts to deny its possibility. On the other hand, pure adsorption phenomena have also been so well established that the colloidal viewpoint of protein reactions appears to be definitely settled. It is quite likely that both colloidal and molecular reactions occur simultaneously in protein solutions and that one or the other dominates

when conditions are more favorable to it. Gelatin in very dilute concentration and in hot solution is very probably molecularly dispersed. In more concentrated and cold solutions, molecular aggregates or colloidal particles certainly exist. The former solution would show molecular (valence) reactions; the latter, colloidal (adsorption) reactions.

Jacques Loeb held the classical (molecular) viewpoint in regard to all protein reactions and attributed the apparent colloidal behavior of proteins to a

. . . failure to measure the hydrogen-ion concentration of the protein solutions, which happens to be one of the main variables. When the hydrogen-ion concentrations are duly measured and considered, it is found that proteins combine with acids and alkalies according to the stoichiometrical laws of classical chemistry and that the chemistry of proteins does not differ from the chemistry of crystalloids.

In addition to maintaining that the physical properties of proteins, their viscosity, their isoelectric point, etc., are determined simply and solely by the hydrogen-ion concentration, Loeb stated further that proteins form true solutions, *i.e.*, molecular and not colloidal dispersions; that they exhibit no selective adsorption; and that, therefore, there are no Hofmeister (lyotropic) series of ions. Loeb was partially right and partially wrong. Gelatin may be molecularly dispersed when in hot and dilute solutions. On the other hand, the colloidal, micellar structure of cellulose, if not of gelatin, has been established beyond all reasonable doubt.

As for the disappearance of hydrogen from hydrochloric acid when gelatin is immersed in it, the question cannot be definitely answered, *i.e.*, whether the hydrogen is removed by adsorption (colloidally) or by chemical union with the protein to form a salt (by a primary valence bond). It appears that some proteins do form salts with acids and bases, while others do not. Whether they do or do not may rest on whether or not there are exposed any free amino or carboxyl groups. The water solubility of albumin suggests an abundance of such free groups. The poor solubility of globulin suggests an absence of such free groups. In the first case, one would expect stoichiometric behavior; and in the second, colloidal behavior.

Evidence in support of the Hofmeister series is now so abundant (with pH control) that their existence can no longer be ques-

tioned. As for the importance of the hydrogen ion, Loeb was right in calling attention to its dominating influence, but as Kruyt says, while the hydrogen and hydroxyl ions have quantitatively special functions, other ions play an important role. It is, therefore, a complete misrepresentation of the true relationships to consider that, in contrast to all other ions, the hydrogen ion plays an all-determining part. Kruyt's statement is particularly true for higher concentrations. It is now generally conceded that the hydrogen ion plays a dominant role when all ions are in dilute concentration but that at higher concentrations other ions play an equally significant part.

IN THE LIVING WORLD

Wolfgang Pauli, in his book "The Colloid Chemistry of the Proteins," says:

There can be no doubt as to the central position of the proteins in the organization of living matter. They alone display the specific properties of life. Distinctions observed, not only between different kinds of organisms but often between individuals of the same kind, reappear on chemical investigation as variations in the respective proteins. The proteins are capable of showing a diversity and fine gradation both in chemical structure and in physical modification to an extent which is lacking in any other class of substances.

The foregoing statement finds reflection in the work of Wrinch, who has put forward a model of a chromosome as an aggregate of polypeptide protamine molecules in association with nucleic acid. As the number of ionized groups possessed by a nucleic acid molecule varies with pH, the chromosome micelles, or protamine nucleate aggregates, will be capable of different configurations for different pH values.

From time to time, emphasis is laid anew on substances other than the proteins as the most fundamental of protoplasmic constituents. Thus, the fact that chromosomes consist in large measure of nucleic acid tends to indicate that this substance is a very significant one in life. The chief rivals of the proteins for first place among the constituents of protoplasm (aside from water) are the fats and fatlike substances such as the sterols and the phosphatides (the so-called lipoids). While the fats do play an important role in vital processes, they seem to serve essentially as nutrient matter or sources of energy rather than as part of the

mechanism itself, though new evidence tends to show that they also serve in the latter capacity (see page 466).

The importance that enzymes, hormones, and the like have assumed led Pauli to modify his statement that the proteins alone display those specific properties that we term life, by saying that the protein plays the more passive part as carrier of the more active vital substances (which may themselves be protein). This is, in a sense, true; but if we compare the concept with that generally held for enzymes, we find that of the two components—enzyme and coenzyme—of which enzymes are presumed to consist, both are equally important; the one cannot function without the other; furthermore, the carrier is specific.

Before we continue with the discussion on the fundamental nature of proteins in living matter, it may again be said (pages 9–10) that many biologists prefer not to regard any one substance in protoplasm as the most fundamental and thus designate it as *the* ultimate living substance. In saying that the proteins “alone display the specific properties of life,” we do not thereby imply that they display all the properties of life nor that they can display even a major part of these properties when alone. Protoplasm is a living *system*. The proteins are the chief constituents of this system, but the system is alive only when water, fats, salts, etc., are also present.

Among the properties characteristic of protoplasm that are due to its protein constituents is coagulation.

Coagulation.—In the past, coagulation has been regarded as incompatible with life, but we now have in living matter a number of very interesting cases of what may be termed reversible coagulation. The idea of the reversible coagulation of proteins is also new chemistry. “When you can *unboil* an egg” has been humorously used by chemists as an expression of the impossible. But apparently it has been done. Living matter has been doing it ever since life began. Dormancy in seeds, over a period of years, probably involves the coagulation of certain protein constituents of protoplasm; it must therefore also involve the reversal of these coagula at the time of germination through the agency of enzymes.

One cannot always be sure whether coagulation or merely an increase in viscosity involving other changes has taken place; thus, the experiment of Bayliss, in which he gave an amoeba an

electric shock, was an example of a pronounced increase in the consistency of protoplasm, but whether gelatinization, coagulation, or an increase in viscosity involving neither of these changes occurred cannot be said. Cessation of the Brownian movement of the protoplasmic particles indicated a change toward a more viscous state. Too severe an electric shock caused a definite and permanent gelation (coagulation) and death. A mild shock was followed by solation, slowly accomplished by the amoeba itself after the current was released. The phenomenon may have been a pure thixotropic one, identical with that occurring in gels. Freundlich and Rawitzer have shown that an electric current will bring about the gelation of a thixotropic sol (Karrer has shown that a current may have the opposite effect and solate a gel). The change in consistency involved in the locomotion of an amoeba is, according to S. O. Mast, simply a rhythmic solation and gelation of the protoplasm. All such changes probably do not involve coagulation. A more convincing case of true and reversible coagulation in protoplasm is that resulting from the addition of acid. M. A. van Herwerden finds that, normally, no structure is visible in the living nuclei of the mesenchyme and nerve tissue in the tail of the living tadpole, nor is a nuclear membrane to be seen (it is often impossible to locate even the nucleus); but if the tadpole is put into water that has been acidified (0.05 part of acetic acid to 100 parts of water), the nuclei assume a clearly defined contour and a granular structure, and return to normal when placed in pure water; they have been reversibly coagulated. If a cell is dividing, the acid renders the spireme or the chromosomes visible. Other structures, such as mitochondria, invisible before, appear in the surrounding cytoplasm. If the tadpole is now placed in pure pond water, the structures described soon disappear. The acid has produced a reversible coagulation without any apparent or permanent ill effect on the protoplasm.

The gelation of protoplasm by injected salts is another interesting case. Kerr has shown that when a calcium salt is injected by micropipettes into the root hairs of the aquatic plant *Limnobium*, the salt may cause the protoplasm to form an irreversible coagulum or a reversible jelly or both, in different regions of the same cell. Coagulation (irreversible gelation) always takes place at the point of injection where the calcium salt is most

concentrated and may occur elsewhere. Gelatinization (reversible gelation) of the protoplasm takes place at a more distant point where the concentration of the salt is not too great. In Fig. 51, coagulated protoplasm is to be seen at the point of injection and in the tip of the root hair (a bridge of protoplasm is formed in the center between two vacuoles). Where irreversible coagulation is slight, the cell may fully recover in twenty to thirty minutes through solation of the reversible protoplasmic jelly. As the latter disintegrates, bits of it are carried in the protoplasmic stream.

The Coagulation of Blood.—Blood clotting is a case of pure coagulation and probably the event that gave rise to the term. It is nature's way of preventing excessive loss from a wound. Mere contact with air is sufficient to accomplish it, and the process is irreversible in the strict sense.

While blood clotting must be regarded as an example of coagulation, its mechanism appears to be wholly distinct from any of the foregoing processes, such as suppression of the surface potential by ions, mutual discharging of colloidal particles, heat, dehydration, and denaturization (decreased solubility due to adsorption). (Fibrinogen is very readily coagulated by heat, at as low a temperature as 56°C., but the natural coagulation of fibrinogen in blood is not by this method.) Blood consists of red corpuscles, white corpuscles, and platelets in suspension in a plasma containing fibrinogen and serum proteins. The white corpuscles are alive. Whether or not the red corpuscles and platelets are to be so regarded is an open question usually answered in the negative. Through the work of Morawitz, Bordet, Howell, Eagle, and others, it has been definitely established that at least four substances are actively concerned in blood coagulation—fibrinogen, calcium, blood platelets, and an unknown plasma substance, termed *thrombogen*, or *prothrombin*. The first—fibrinogen—is the substance coagulated, being converted into fibrin. The other three interact to form *thrombin*, the actual coagulating substance; thus, the soluble protein fibrinogen is converted by thrombin into the insoluble protein fibrin, a fibrous coagulum.

The function of calcium in this process is unknown. It may be an intrinsic active part of thrombin. The fact that thrombin to which oxalate or citrate has been added is still active does not

exclude this possibility. The molecular concentration of the active substance may be so minute that enough free Ca^{++} is still left in solution to form thrombin. Calcium is not a stoichiometric constituent of the fibrin formed by the action of the thrombin upon fibrinogen. (It is interesting to note that the action of calcium is specific and that while other bivalent cations such as barium and strontium have an effect, it is very slight compared with that of calcium.)

The platelets appear to liberate a substance that greatly hastens coagulation. Whether or not they are indispensable is unknown. Although coagulation may take place even when no platelets are present, this may be due to the presence of disintegrated platelet material. The blood of birds has very few platelets; its plasma coagulates poorly. The addition of platelets from the blood of any other animal hastens coagulation. When blood is withdrawn from the body, the platelets disintegrate rapidly and in so doing presumably set free a substance that functions as a catalyst in hastening coagulation. In the human disease known as *purpura hemorrhagica*, there is platelet deficiency. Coagulation occurs but is retarded. Further evidence is to be had in that clot retraction, for which platelets are essential, does not occur in purpura.

The fourth substance actively involved in blood coagulation, being the third of the trio which, combined, constitute thrombin, is the unknown plasma substance which has been termed thrombogen, or prothrombin. Little is known of its action other than that it appears to be the true precursor of thrombin activated by calcium and platelets.

The mechanism of blood coagulation in general is regarded by some as comparable to the coagulation of proteins by enzymes, such as the coagulation of milk by rennin.

Human beings known as bleeders suffer from hemophilia, a condition preventing the coagulation of blood when it reaches the surface of a wound. It is a heritable quality transmitted solely by women, in whom it is suppressed (as a recessive character); only men suffer from it. Its cause is unknown, but it may be due to stability of the platelets, thus preventing the liberation of their active coagulating principle; possibly it is due to retarded activation of prothrombin.

The speed at which blood coagulates varies in different animals. The blood of geese coagulates very slowly, even more slowly than that of hemophiliacs. Glycolysis, or the splitting of glucose into lactic acid, and blood coagulation are coupled reactions; *i.e.*, glycolysis (decrease in sugar or increase in lactic acid) and coagulation are rapid in normal men (6 min. for coagulation), slow in hemophilic men (180 min.), and very slow in geese (252 min.).

We now come to the evident question, Why does blood not coagulate in the body, as it contains all of the four constituents necessary for coagulation? The suggested cause of hemophilia is again a possible answer here, *viz.*, that the platelets do not break down when in the body. It has also been suggested that some substance normally in the blood, and continuously supplied to the blood when in the body—possibly heparin—functions as an anticoagulant. This problem and others, such as the fact that blood does not coagulate when withdrawn into paraffin-coated vessels, remain for future workers to solve. Acidity and alkalinity, so often found to be contributing if not primary causes of physiological processes, apparently are not significant factors in blood coagulation. Eagle and Baumberger find no marked change in pH during the coagulation process.

Blood coagulation does not appear to rest upon a strictly colloidal property (*e.g.*, instability) such as is responsible for the coagulation of lyophobic suspensions. The question, therefore, arises, What is its physical nature? Little can be said. It is not known whether fibrin (the coagulum) is a product of fibrinogen (the substance coagulated) or whether coagulation involves simply an intramolecular rearrangement. Either of these is possible in the light of our present knowledge. Although still controversial, the bulk of evidence favors the theory that thrombin is an enzyme activating the transformation, rather than a substance that combines with fibrinogen to form fibrin.

Natural Anticoagulants.—The clotting of blood is a desirable feature for those whose blood is involved; but for animals seeking the blood of others, clotting is objectionable. The leech, the vampire bat, and the lamprey feel this way about it. They live upon the blood of animals, the flow of which would stop if they did not have some means of preventing coagulation. The leech and the vampire bat produce a secretion which they pour into the wounds that they make. A secretion from the buccal glands

of the lake lamprey when mixed with the blood of a bony fish, such as the lamprey often feeds upon, prevents the coagulation of the blood. The secretion is effective in preventing or delaying the coagulation of human blood when the two are artificially mixed.

Anesthesia.—Some sixty years ago, Claude Bernard advanced the hypothesis that anesthesia involves the reversible coagulation of the colloids of the sensory nerves by the anesthetic. The hypothesis was rejected because it was (until quite recently) thought that a coagulum is incompatible with life. This criticism has been met by showing that the amount of anesthetic (alcohol) necessary to coagulate a colloid may be quite small and that under these conditions the coagulation is reversible. The theory of Bernard has been revived and given newer support by Bancroft. That we lose consciousness under an anesthetic because the protoplasm of our nervous system is coagulated, mildly and reversibly, is a plausible hypothesis. Severe, irreversible coagulation means death.

There are numerous other hypotheses of anesthesia none of which is fully accepted.

Cellular Agglutination.—Bacteria in culture are often to be seen clumped together in isolated groups. They have *agglutinated*. This means death unless they are soon redispersed. The cause of bacterial agglutination is a reduction in their surface potential, not charge (see page 372)—at least so it would seem from the work of Northrop and Kunitz, who have shown that certain bacteria agglutinate at a definite minimum electric potential of 11 mv. (page 376). The relationship between potential and agglutination is specific and not general; that is to say, each species has, within limits, its own particular minimum potential at which it agglutinates.

The agglutination of bacteria plays a significant part in the defense mechanism against disease. It is not in itself sufficient to render pathogenic bacteria inert, for the attack on bacteria within the body is one of chemical neutralization (page 502); that is to say, antitoxins function through chemical union with toxin, but agglutination is usually associated with chemical neutralization and thus may contribute.

There is a very remarkable type of cellular agglutination which does not belong strictly to the purely physicochemical

forms of coagulation that we have so far considered. When bacteria agglutinate, they play no active part as *living* particles; the mechanism involved is identical with that which brings about the aggregation of nonliving particles. The agglutination or assembling of certain cells, however, rests, at least in part, upon their own vital activities. This is the case of the extraordinary "heteroagglutination" of dissociated sponge cells described by Galtsoff. If cells of the sponge *Microciona prolifera* are artificially separated, they reunite by amoeboid movement. This is not in itself extraordinary, as cells aggregate to form colonies or unite to form tissues; but among the individual cells of the seven kinds that make up a sponge, each joins its *own* kind. Chemical distinctions in the cells are possibly responsible for this each-unto-his-own reunion. When the cells of two different species are mixed, there is no mingling of the two in the reorganization process. Such an agglutination or assembling of cells may have certain physical and chemical properties in common with ordinary coagulation processes, for in the latter, while there is no superficial evidence of selective arrangement, as in the case of the cells of a sponge, there is evidence that the action of protein (blood) coagulants—in particular, the specificity of antigens and antibodies—is due to a definite arrangement within the molecules analogous to that of the lattice structure of crystals. This structural orientation may be responsible for the combination of a certain antigen with a certain antibody, as will be seen in a moment; it may also be responsible for each cell of a disorganized sponge reuniting only with a cell of its own kind.

Protein Individuality and Specificity.—Emphasis on the proteins as representing the most significant of the constituents of protoplasm, in that they among all classes of compounds display a greater number of the specific properties of life, suggests that organisms are what they are because of the proteins that they contain. This may, in a broad sense, be true, and it seems probable, in the light of Abderhalden's and of Leathes's estimates of the almost inconceivably great number of permutations (amino-acid orientations) in proteins, that every species, if not every individual, has its own specific protein or protein group. It has already been suggested that important as the proteins are, they may serve primarily as carriers of other even more

significant substances (page 492). This does not alter the fact that the proteins are probably specific—specific for the substances with which they are associated, specific for a given species, and specific for individual differences in a species. The following are other better authenticated examples of protein *individuality*—a preferable expression for what we here have in mind, because while both individuality and specificity are based on stereochemical congruity, protein specificity implies, in immunology, an *elective* chemical affinity (*e.g.*, the reaction between antigen and antibody).

One individual's susceptibility and another's immunity to poisons, bacteria, proteins, and toxins in general are problems in protein individuality, as is also the fact that the sperm of only the same species (and not even always then) can fertilize an egg; that only certain animals can save themselves from certain diseases through the production of an antitoxin which counteracts the toxin (*e.g.*, the horse in protecting itself against diphtheria); that the geranium is susceptible to the plant-gall bacillus, and many other plants not; that the chestnut is susceptible to a blight caused by the fungus *Endothea parasitica*, while other trees are not; that man is immune to the distemper of dogs and highly resistant to the foot-and-mouth disease of cattle; that the hair and feathers of most domestic animals are poisonous to some persons but not to others; that nearly all people are sensitive to one or more of 150 common foods among which strawberries and eggs are the chief offenders.

Substances other than proteins may at times be the cause of an immunity or sensitivity; consequently, *chemical* rather than protein individuality is the more exact expression. Fats and carbohydrates occasionally appear to be responsible (*e.g.*, in the case of the capsular carbohydrate of pneumococci).

Immunology embraces the problems that we have been considering. It is the study of the resistance of the living organism to pathogenic or disease-producing agents. In addition to a natural or inherited immunity, there is an acquired immunity. We become immune to measles after the first attack, but we do not inherit the acquired immunity that our parents developed. Acquired immunity can be obtained in three ways—by getting a mild case of the disease ourselves, by vaccination, or by taking advantage of the immunity acquired

by some other animal. The first method—that of active immunization—is quite successful in cases of smallpox and typhoid. Smallpox may also confer immunity against other similar diseases such as cowpox; or, what is of more importance medically, cowpox confers immunity against smallpox. Passive immunization has had its greatest triumph in the case of diphtheria, for which serum from an immunized horse is an almost certain cure. Where acute diseases, for example, those of children, such as measles, mumps, chickenpox, and infantile paralysis, do not attack animals, the immune serum must be had from human beings. Success has attended the prophylactic injection of serum of convalescents from these diseases.

Practical immunology has to do with the administration of a substance that will combat a disease, that is to say, will lead to the neutralization of the toxic substance in the patient. The toxic substance may be living, or nonliving, *viz.*, bacteria, a virus, or an organic compound (not produced by bacteria). There are many diseases of this last type, hay fever being one. The agent causing the disease is a *toxin*, or *antigen*; the substance produced and reacting with (counteracting) the antigen is the *antitoxin*, or *antibody*. The production of an antibody, whether in the natural course of living or through artificial injection of an antigen into an animal, is known as *immunization*. An antigen need not be poisonous; more precisely defined, it is any agent that will bring about the production of an antibody. The substance administered may be a *vaccine* or an *antitoxin* (immune body). A vaccine is composed of the "attenuated," or killed, pathogenic agent itself; while antitoxins are substances (possibly proteins) produced by an animal which has previously had the disease in question. The animal may have acquired the disease naturally or, ordinarily in immunological practice, from vaccination or the injection of the disease-producing agent.

Special names have been given to certain immune substances, or antibodies, and their corresponding inciting substances, or antigens. Some of these are

Antigen	Antibody
Enzyme.....	Antienzyme
Bacterial exotoxin.....	Antitoxin
{ Bacterial protein.....	Agglutinin }
{ Vegetable and animal protein.....	Precipitin }
Animal venom.....	Antivenom

Of late, it has become possible to render an antigen nontoxic without robbing it of its antigenic capacity to incite antibody formation. Such nontoxic antigens are known as *toxoids*. For example, diphtheria bacilli can be treated so that they are still antigenic, *i.e.*, still have the capacity to cause antibody production, but are now nontoxic and so cannot cause diphtheria. The toxin is now a toxoid. Toxoids may be injected directly into human beings without the intermediary of an animal, because while we ourselves are unable to produce a diphtheria antitoxin in sufficient quantity fully to counteract the toxin when attacked by the disease directly, we can and do produce antitoxin adequately when injected with toxoid.

Most immunization is for the purpose of prevention rather than cure; thus, vaccination for smallpox and inoculation for typhoid are done to healthy people in anticipation of the disease. The treatment is prophylactic. Once within cells, the disease-producing parasites cannot be easily reached by injected serums, while if the prophylactic serum is already present in the body fluids, then the parasites will be killed when they enter the body, as they are intercellular before they can become intracellular. Some few antitoxins, such as those against diphtheria and tetanus, act as cures but only when administered in the very early stages of illness.

Human sensitivity to foreign proteins, such as those in the serum of horse blood, is an example of that important branch of protein individuality known as *allergy*.

We come now to a more detailed consideration of the question how far immunity and allergy are problems in protein chemistry; in other words, are the proteins of the cell and of the body fluids the responsible substances?

It was formerly thought that no substance other than a protein could be an antigen and that, on the other hand, nearly every known protein is antigenic (to some animal). Thus, Wells wrote that immunological specificity must depend in large part upon differences in proteins, for antigens are usually, if not always, proteins. While Wells realized that other substances might on occasion be responsible, he added that if we turn to one of the most likely of such other substances, the lipoids, we find Levene saying that he has failed to discover any distinction between lipoids derived from different tissues of the same species. On

theoretical grounds, it is possible that lipoids exhibit specificity. Carbohydrates had not, at the time when Wells wrote (1929), been found to be antigenic, though he recognized the fact that they are capable of exhibiting immunological specificity. It is now known that certain polysaccharides are, and that lipoids may be, antigens.

The evidence in favor of a protein nature of antibodies is reasonably convincing. Precipitation, cataphoresis (isoelectric point), stability, and destruction (with heat, alcohol, etc.) all show a close correspondence between antibodies and proteins. The antibody protein is presumably a globulin fraction. Experiments by S. Mudd on cataphoresis (bearing on the problem of phagocytosis) are reasonable evidence of the globulin nature of antibodies.

Antigens and antibodies being recognized as (mostly) protein, the next problem has to do with the mechanism by means of which the former are rendered nontoxic by the latter. The question is a basic one in protein specificity and first received the serious attention of the German chemist Paul Ehrlich, to whom much of the early development of immunology is due. He represented the molecules of the antigen and the antibody as geometrical figures which fit into each other like a key into a lock. Restated in other terms, the hypothesis of Ehrlich implies that immunological reactions between proteins are due to arrangements of the constituent parts (amino acids) of the molecules. The arrangement responsible may not be of the molecule as a whole but of a *determinant group* in the antigen and a *receptor site* in the antibody. (These terms are simply convenient expressions for specific molecular orientations; chemically, they would be called atomic groups, radicals, or side chains.) As Marrack puts it, specificity depends upon a specific configuration of atoms representable by chemical formulas. Such a hypothesis of immunological reactions is a confirmation of Ehrlich's views. Those who oppose the lock-and-key hypothesis regard the combination between antigen and antibody as one of adsorption. But as adsorption bonds may apparently be anything from primary valence to a loose electromagnetic attraction, it would be difficult to distinguish between adsorption and an interlocking of atomic structures.

Kinship.—Few, if any, subjects in biophysics and biochemistry are more fundamental than that of the chemical basis of kinship. Plants or animals that are closely related are quite likely to have a similar chemical constitution. If we attribute to chromosomes all the responsibility for kinship between organisms, we are but using a collective term for the substances of which chromosomes are made. Chromatic material appears to be primarily responsible for kinship, but other substances outside the chromosomes may be, if not responsible for, then at least an indication of relationship. Among these latter substances are those in the body fluids, particularly the blood. Blood specificity has become a large chapter in immunological work. The older medical viewpoint, with its complex and confusing nomenclature and somewhat vitalistic tone (for specificity was thought to be too extraordinary to be due solely to the laws of physics and chemistry), has now given way to a strict physical and chemical interpretation.

Among the first significant contributions to modern views on specificity in organisms was that of Landsteiner on blood types. Human beings have blood that belongs to one of four groups (the fourth may be a mixture of more than one). The groups are determined by agglutination tests. So definite is the type that it is now used to establish parentage and relationships both in theoretical biological studies on evolution and heredity and in practical criminology and illegitimacy.

In early work on immunity, it was noticed that species react similarly in respect to their immunity from reinfection. Their proteins should, therefore, show similar (precipitation) reactions when combined with antibodies. This fact led a number of workers to study the immunological reactions of species with the hope of determining phylogenetic or family relationships. Among those active in this field were Landsteiner and Nuttall on animal relationship and Mez on plant relationship. When a vegetable or animal protein (antigen) is injected into an animal (of a different species), a precipitating antibody is formed. The intensity of the precipitin reaction between these antibodies and various plant or animal proteins gives an indication of the nearness of their relationship. On this basis, Nuttall obtained the following results:

Blood Serum from	Intensity of Precipitin Reaction
Human beings.....	100
Anthropoid apes.....	100
Common monkeys of the old world.....	92
Capuchins and spider monkeys of the new world.....	78
Marmosets.....	50
Lemurs.....	0

(The number 100 indicates a reaction identical in intensity with that of the original serum used in immunizing—in this case, human blood. The table, therefore, indicates the nearness in relationship of the lower animals to man.)

Landsteiner and Miller have shown a similar relationship between man and the anthropoid apes. Manoiloff found that the blood serums of cattle and horses can be distinguished by precipitin reactions. He believes that the distinction lies in the albumins.

The tame rat, *Mus rattus*, and the wild one, *M. norvegicus*, do not cross. When artificially inseminated, the female gives sign of pregnancy but later returns to normal; yet the two animals are so closely related that they were formerly thought to be of the same species. Such instances are probably due to protein incompatibility, but the cause may also be purely genetical, one or the other germ cell possessing what is known as a lethal factor (or deadly gene) which prevents growth of the embryo. However, as both adults may have progeny with other mates, protein incompatibility is the likely factor.

The serological studies of Mez on plant relationship permit the construction of a plant family tree which corroborates almost fully the older classification based on the usual taxonomic (morphological) characters. Where differences in results by the two methods exist, the correct relationship is just as likely to be shown by the newer serological reaction as by the older anatomical evidence. It is of great significance to the science of evolution and phylogenetic relationship that a purely chemical basis of classification should so well support a purely anatomical one. Relationships between plants established by serological methods hold well for families but not so well for genera and not at all for species. This is due simply to a lack of delicacy in the technique; species differences in proteins must, of course, also exist.

The statement is occasionally made that protein specificity is not "biological" but chemical, by which is meant that the reaction is not necessarily associated with life; *i.e.*, living material is not needed in order to establish a plant or animal relationship on the basis of immunological reactions; old serum gives the same result as fresh serum.

The work of Moyer (page 386), in which plant relationship between species of *Euphorbia* was established on the basis of rate of cataphoretic migration (mobility curves) and isoelectric points, appears to rest, in part at least, on the protein nature of the covering of the latex particles, but Moyer is careful not to assign all responsibility to the proteins. The constitution of the covering of the latex particles is a chemical criterion of relationship, but that this covering is completely or always protein is not conclusively proved—in fact, there were observations against it; often no test for protein could be obtained; the latex particles of numerous species pass readily into oil, suggesting little or no protein on the surface; isoelectric points were sometimes too low for protein. These facts indicate that other substances may be responsible. Likely ones are the sterols or the higher alcohols (*e.g.*, resin alcohol, $C_{16}H_{47}OH$; or cetyl alcohol, $C_{16}H_{33}OH$). The sterols especially have come very much to the fore of late as significant substances in biological systems (page 466).

An interesting addition not only to protein specificity as the determining factor in species relationship but also to methods ascertaining these is the work of Svedberg on sedimentation constants, molecular weights, and isoelectric points of respiratory proteins. He found that sedimentation constants of proteins determined by centrifuging are of the same value within a well-defined animal group. Thus is biological kinship between species related to the molecular weights of their proteins.

Heredity.—Basing biological kinship on protein specificity is a step, and a very definite one, toward a chemical interpretation of heredity. A further step could be taken by enlarging on the subject and raising it to the dignity of a chapter, but, attractive as this would be, it would be premature. We might, however, well look for a moment in this direction and realize that the day may come when not only the laws of heredity but its basic mechanism as well will be stated in terms of physics and chemistry, just as the mechanism of muscular action and nerve con-

duction are now no longer problems in biology or medicine but problems in physics and chemistry.

Speculations on the physical and chemical nature of the hereditary substance or particle (the gene) are based on the main thesis of this chapter, *viz.*, that protein is the primary vital substance. Such a conclusion in regard to heredity is reached by Demarec. He views the gene as a protein complex or possibly a single protein molecule.

Estimations by Muller of the size of the gene (highly speculative in themselves, for we are not yet certain that the gene is a definite individual particle) place it at 50 m μ . This is ten to twenty times the size of large protein molecules. Gowen and Gay place the maximum gene volume at 1×10^{-18} cm 3 .

CHAPTER XXVI

REGULATORY SUBSTANCES

The activities of cells are in great measure determined by specialized substances which appear to be of a complex nature. Perhaps they appear so because of limitations in our knowledge which preclude an as yet definite classification of them with the salts, carbohydrates, fats, or proteins. These specialized substances are the *enzymes*, *hormones*, *vitamins*, and *pigments*. Certain of them, or the responsible constituents within them, have been found to be very simple compounds—even single elements—consequently, the presumably complex substance (*e.g.*, a hormone) may be simply an element (*e.g.*, boron) or a radical (*e.g.*, the sulphhydryl radical SH).

Enzymes, hormones, vitamins, and pigments serve as determiners of the activities of single cells and of intercellular activities of the organism as a whole. Regulatory substances thus control and harmonize body functions. While protoplasmic in origin, they usually enter the body fluids and there serve their purpose as organizers. Through them the cells of the body become more intimately associated. Without them, there would be no organized whole. While we shall consider them primarily as regulatory substances of the entire living body, it should be remembered that they are concerned just as much in determining and controlling the activities of a single cell.

ENZYMES

Enzymes are important nutritional growth-producing and activating substances occurring in cells and organisms in great numbers. They are regarded as catalysts accelerating metabolic reactions, such as the conversion of proteins, fats, and carbohydrates into their simpler component parts. Enzymes are often resorted to in explanation of reactions even though the evidence of their presence may simply be the knowledge that *something* must be responsible for a certain activity. We believe that chlorophyll contains something that is responsible for the syn-

thesis of sugar from carbon dioxide and water; we call this something an enzyme and name it *chlorophyllase*. We assume a great deal when we do this. But in many other instances the enzyme is definitely known, as in the case of invertase (a sugar-splitting enzyme) and pepsin (a protein-splitting enzyme).

The enzymes are classified either according to the kind of substances that they break down or the way in which they do it. There are (1) hydrolytic enzymes, including (a) the esterases (lipases) or fat-splitting enzymes, (b) the carbohydrate-splitting enzymes (cellulase attacking cellulose, and maltase attacking maltose), and (c) the protein-splitting enzymes (pepsin and trypsin—these were known before the ending *-ase* was adopted for enzymes)—(2) oxidative enzymes, including (a) the oxidases, (b) peroxidases, (c) the zymases (splitting hexose sugars such as glucose and fructose), and (d) the catalases. The oxidases and peroxidases are enzymes of particular biological significance.

A few enzymes have been obtained in pure crystalline form. Northrop has crystallized trypsin (the pancreatic protease), pepsin, and urease.

Willstätter believes that enzymes are not single substances but are always associated with a colloid carrier, *i.e.*, a protein that carries the active principle, or enzymatic component. The two constitute an adsorption complex.

Enzymes have long been thought to be highly specific (*i.e.*, a definite enzyme for every specific reaction in the plant and animal body). Pasteur believed them to be vital substances capable of being produced only by the living cell. Many of them are proteins. Enzyme activity and enzyme specificity thus become the reactions of highly specialized protein groups. The degree of specificity of enzymes is uncertain. Some, such as invertase, which splits sucrose into dextrose and levulose; and catalase, an oxidizing enzyme, which splits peroxides into oxygen and water, seem to be highly specific, while others, such as the lipases, which split neutral fats into glycerin and fatty acids, attack large groups of related substances. With a change in the nature of the medium there may also be a change in the nature of the reaction; *i.e.*, the enzyme may function as a catalyst in more than one way. R. B. Harvey, therefore, states that it is not necessary to assume the presence of a separate specific enzyme for each process. Indeed, certain "enzymes" (*e.g.*, the oxidases)

are best regarded as types of reactions rather than as actual chemical entities.

Present-day research on the enzymes is concerned primarily with establishing their chemical nature, their mode of action, and their specificity. Their mode of action has been suggested (page 502), though it is problematical. Linderstrøm-Lang has demonstrated a regulatory influence of dipeptidase. He finds that it is most concentrated in the meristem (the center of most active cell division) of the root and rapidly falls off away from the meristematic region. Dipeptidase is, therefore, an activator of mitosis and thus through its presence or absence becomes a regulator of cell division, which is the basis of (proliferative) growth.

HORMONES

Endocrinology.—The name *hormone* was first applied to the secretions of glands. The presence of substances that control growth and regulate body functions had previously been demonstrated by the botanist Sachs. All forms of cellular secretions become hormones in so far as they function as regulatory substances. Secretin, from the mucosa of the duodenum, was the first hormone to be so named. Insulin, from the pancreas, is one of the best known hormones because of its function in maintaining the normal metabolism of carbohydrates in the body; artificially injected into the body it becomes of great medical value in the treatment of diabetes.

Endocrinology is the science of the function of the endocrine glands—the ductless, or internally secreting, glands—it constitutes one of the most important fields of study in modern medical research. One might almost say that what we are is determined by our chromosomes and what we do, by our hormones. Our bodies contain at least 12 recognized endocrine glands; they include the pineal, pituitary, thyroid, parathyroid, adrenal, ovarian, and testicular glands. Each elaborates a specific active principle, or hormone. Hormones control definite chemical reactions in the body, functioning as catalysts.

One after another, these formerly mysterious substances are being isolated, crystallized, and synthetically produced in the laboratory, even on a commercial scale. The capacity of the thyroid gland to maintain regulated growth in the body and to

control metabolic rate has long been known. Now we know the exact molecular structure of the substance thyroxin, which is responsible for these effects.

We are here interested primarily in the secretions of cells other than those which are aggregated into specialized tissues or glands. Among the first of these to be studied was the "organizing substance" isolated from embryos by Spemann.

Chemical Embryology.—Among growth-regulating substances are those responsible for the proper development or organization of an embryo. Why an embryo grows into this or that kind of animal is determined by its chromosomes, but for normal development certain growth regulators or organizers are necessary. The first definite proof of the presence of such substances came from the work of Spemann, who, in 1918, found that the region of the dorsal lip of the blastopore is an *Organizer*, or differentiator. On grafting a piece of it to another embryo of different species, he found that it caused cells, which would otherwise have gone along quite other lines of growth, to develop into a secondary embryo. The organizing property is not characteristic of the tissue as such (of the blastopore lip); other tissue will do as well if it has been in contact with the organizer. Obviously, some chemical substance is responsible. Further, the regulating substance is not specific; that from any species (so far as tried) will serve to stimulate and direct the cells of another species.

The next task in the work was the isolation of the organizing substance and a determination of its chemical nature. J. Needham has been responsible for a great deal of this work, though others have contributed much. Mangold found that the organizer retains its activity unimpaired for a long time. It may be boiled, narcotized, crushed, dried, or frozen without loss of its inductive power. It appears definitely to be an ether-soluble substance, possibly lipoidal in nature; it may be a sterol or a hydrocarbon; if the latter, it is comparable to the cancer-producing hydrocarbons. Certain other substances, however, function as organizers, *e.g.*, thyroxin.

Plant Hormones.—Plant-regulating and growth-stimulating substances were first referred to by Sachs and later more extensively studied by Haberlandt, who was particularly interested in wound-healing hormones. The healing of wounds in animals as well as in plants and the regeneration of body parts (*e.g.*, in lower

organisms) involve the production of growth-provoking or wound-healing hormones.

The most recent work on plant-growth hormones is that of Kögl, Thimann, and Went. Previous to them, Paál had shown that the tip of a growing root contains a growth-stimulating substance; if the tip is removed, growth ceases. Injury is not responsible, for if the severed root tip is replaced, growth continues; but if a thin sheet of tinfoil or mica is placed between tip and stump, no growth results. The same tip is not necessary; one from another root will do as well, but a piece out of the base

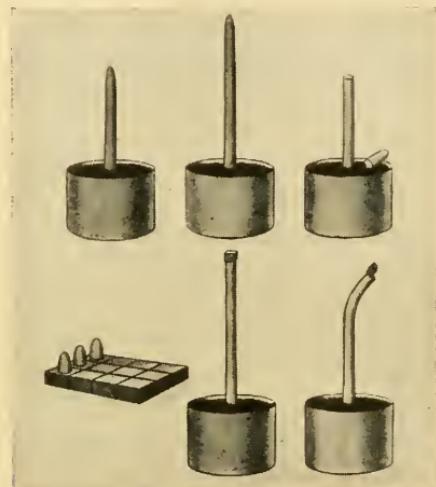


FIG. 178.—Extraction of the growth hormone from root tips placed on agar blocks (lower left). Continued vertical growth (lower center) or curved growth (lower right) results when the blocks are substituted for the tips. (From F. Went.)

of the root will not do. If the tip is replaced off center, growth is greater on the one side than on the other, and the root curves. Went was able to extract the growth hormone by placing root tips on agar, gelatin, or silica gel and then putting the gel blocks in the place of the root tips; growth continued vertically when the block was centrally placed, and laterally, forming a curve, when the block was placed off center (Fig. 178). The growth-stimulating hormone was isolated by Kögl and named *auxin*; it consists of three forms—auxin *a*, $C_{18}H_{32}O_5$; auxin *b*, $C_{18}H_{30}O_4$; and heteroauxin, $C_{10}H_9O_2N$. The amount in a root tip is extremely small, but it has now been obtained in larger quantities from

human urine, a source of many highly important physiological substances.

The presence of a growth-stimulating hormone may be the explanation of the experiments of Ferguson and Duggar, who found that a high percentage of germination of mushroom spores in culture was always preceded a few days by the germination of one or two isolated spores. As the tube of a single spore, say at the edge of a culture, reaches the central mass of spores, then all begin to germinate. Cultures of spores into which bits of the mycelium (fungus body) are introduced give almost perfect germination in half the usual time. It appears that the germinating spore tube or the mycelium produces a substance that stimulates other spores to more rapid growth.

In General.—If we define a hormone as a substance that coordinates the functions of organs by exciting them to activity, then it is obvious that whether such a substance is a complex glandular secretion about which we know little or a single element such as boron, it is a hormone; and when we learn more about the complex hormone, we may find that its active principle—the catalyst—is a simple group if not a single element. The word "hormone" is a useful one and need not give rise to misunderstanding if we realize that it refers to any catalytic agent in metabolic or other internal reactions. In this sense, it becomes synonymous with enzyme (at least to some of them) and possibly also with vitamin. Opposition to the hormone concept arises particularly in such picturesque instances as the following: The German philosophical biologist Hans Driesch was seated in his garden with a student and observed a hen peck at her chicks which yesterday she had mothered. Driesch turned to his student and remarked, "Sehen Sie, Herr Doktor, die Liebe ist nur ein Hormon." Whatever our reaction to this statement by Driesch may be, it is yet true that no emotion can exist without its corresponding hormone.

VITAMINS

British merchant ships were once humorously referred to as "lime tubs," and British sailors are still called "limies" or "lime juicers," all of which dates back to the day when it became a British law that no British ship should leave port without

so and so many quarts of "lime" juice per man per month of voyage. The "limes" were lemons, and the juice was to prevent scurvy. Today, with modern nutritional methods and shorter voyages, this precaution is not necessary, though scurvy still occurs where fruits, vegetables, and fresh meats are not available, as, for example, in arctic regions. In spite of the knowledge that lemon juice prevents scurvy, it was not until 1897 that the first successful experiment on *vitamins* was done and not until as recently as 1910 that the significance of this experiment was fully understood.

In 1891, Bunge stated that there must be present in milk substances other than casein, fat, lactose, and salts which make milk a suitable food. Six years later, Eijkman found that fowl restricted to a diet of polished rice developed polyneuritis, the analogue of beriberi in man. He then discovered that the coating on rice contained something that prevented the disease. Funk, in 1910, repeated Eijkman's work and gave the word "vitamin" to those specific substances which are essential to normal nutrition and prevent disease. Simultaneous with this work was that of the dieticians who found that purified proteins, carbohydrates, fats, and salts are not sufficient for growth in animals. E. V. McCollum, the foremost worker on vitamins in America, found, with M. Davis, that certain fats, *e.g.*, butterfat and egg-yolk fat, contain some substance that is essential to growth, while olive oil and lard do not contain this substance. Later, Osborne and Mendel added cod-liver oil to the former two. The substance in these fats necessary for normal growth is now known as vitamin A. At least 6 essential vitamins are recognized, and 10 have been named.

The term vitamin may be applied to any substance that is indispensable for normal growth and good health in respect to some special function such as the prevention of a specific disease. Vitamins are distinguished from hormones in that the latter are usually produced within the body, while the former are supplied in the food. The vitamins are lettered A, B, C, etc., and further characterized as fat or water soluble. Though some are abundant in animal tissues, plants are the chief source; for this reason, vitamins have received the name of plant hormones. (The prevailing opinion is that vitamin A is not found in plant tissue; only carotene, the precursor, is so found.)

Vitamin A.—A disease of the eyes known as night blindness and subsequently called by McCollum "xerophthalmia" was by Osborne and Mendel demonstrated to be due to a deficiency in diet. McCollum and Simmonds then found that diets lacking in butterfat cause the disease. The specific substance present in butterfat which prevents the disease is vitamin A. Its absence also prevents normal growth, and so it is known as the "growth substance." (Actually, normal growth depends upon the presence of 30 or more indispensable nutrient substances.) Other important disturbances resulting from the lack of vitamin A are lowered resistance to infections, diseased condition of the kidneys, disturbance of oestrous cycle and lactation, retardation of growth, sterility, etc.

The most potent natural sources of vitamin A are halibut and cod-liver oil, but the vitamin is abundant in milk, egg yolk, butter, carrot, lettuce, etc. It is produced in the body through conversion, by the liver, of carotene, $C_{40}H_{56}$, the yellow pigment in vegetables (particularly carrots and corn), fruits, and butter.

Vitamin B.—Vitamin B holds the historical position of the first vitamin studied which led to the coinage of the word. Its absence usually causes beriberi, common among rice eaters of the orient, although polyneuritis may also result as well as injury to nerve tissues, loss of appetite, and impairment of digestive processes. The chemical constitution of vitamin B is undetermined, although it is known to be a nitrogenous base. Knowledge of its true physiological effects is complicated by the fact that it is usually accompanied by the second component—vitamin G (vitamin B_2).

Vitamin B is widely, though sparingly, distributed in plant and animal material. It is most abundant in yeast and the embryos of grains (wheat, barley, etc.). The vitamin B in cow's milk is insufficient for infants; it must, therefore, be supplemented (by the tenth day).

Vitamin C.—Vitamin C is best known for its antiscorbutic properties. Scurvy appears to be limited to man, monkeys, and guinea pigs. It is most readily produced in guinea pigs by a diet of cereals and bread. The addition of fresh vegetables cures the disease.

Vitamin C is almost solely a plant product. It is abundant in paprika, red capsicum, and lemon and orange juice and is found in cabbage, lettuce, and celery.

Vitamin D.—Vitamin D has received much attention of late because of the serious effect of its absence on children. Rickets results from vitamin D deficiency. This vitamin is the principle directly concerned with maintaining the calcium and phosphorus balance of the blood at the normal level and hence with the calcification of the bones of the body. McCollum first demonstrated the effect of cod-liver oil on a low-calcium and high-phosphorus rickets.

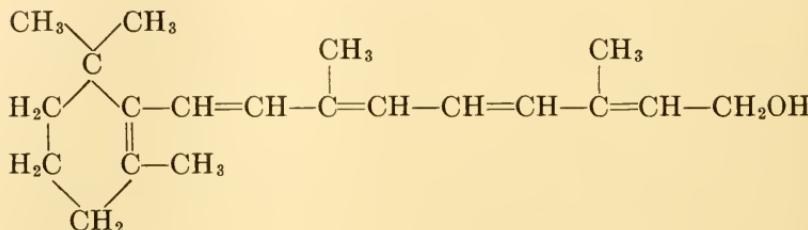
Vitamin D is most abundantly found in cod-liver oil. As an antirachitic agent, this oil has a perfect counterpart in the ultra-violet component of sunlight. Vitamin D is found in some few other animal products (egg yolk, butter, milk) but not in plants. The activation of ergosterol results in the production of vitamin D (see page 465).

Vitamin E.—Animals confined to a certain ration often become sterile in the later stages of reproduction. The embryo dies, usually soon after the twelfth day of fetal life. A definite food requirement is needed by the developing young, and this is now known to be vitamin E. This antisterility vitamin is present in small quantities in animal tissue but much more abundant in plants, its potent source being wheat-germ oil.

Vitamin G.—Vitamin B has been found to consist of two principles, both active in nutrition. The first, or antiberiberi factor, retains the designation B. The second has been termed both B₂ and G. Its presence prevents pellagra, a disease of southern Europe characterized by erythema and nervous and digestive disorders. Vitamin G, or B₂, is widely distributed in plants and is usually associated with B. The richest carrier of both is yeast, although cottonseed meal is the cheapest source of the pellagra-preventive vitamin G as well as of vitamin B.

Chemical Constitution of the Vitamins.—Several of the vitamins have been obtained in the crystalline state, and one has been synthesized. We are thus very near to an understanding of their constitution. The chemical nature of vitamin A is known with a high degree of probability. It is not a sterol, as are vitamins D and E, but rather a phytol-like compound. The formula on page 516, by Karrer, is generally accepted. (There is a suggestion of isoprene structure in the side chain on the beta-ionone ring.) The constitution of a long carbon chain attached to a ring relates vitamin A to certain vegetable pig-

ments, notably carotene. (While carotene has no vitamin-A content, it is a precursor of it and is converted into vitamin A by the liver.) The formula of vitamin A is as follows:



The molecular structure of vitamin D is similar to that of the sterols, which are converted into it through activation by ultra-violet light.

PIGMENTS

Pigments have played but a small part in physiological studies in the past. Only recently, with the finding of the striking relationship between carotene and vitamin A, has an at least partial realization of the nutritional value of pigments been attained. Equally fundamental has been the discovery of Keilin; by spectroscopic observations he proved the existence of three pigments, collectively known as cytochrome, in most living cells. Cytochrome is now recognized as a respiratory pigment of prime importance.

To what extent other pigments are of nutritional or enzymatic value is not yet fully known. For carotene (from carrots and other plants, *e.g.*, from the oil of palms), the problem seems to be solved; its physiological value lies in its capacity for conversion into vitamin A. It is given the formula $\text{C}_{40}\text{H}_{56}$ and is thought to consist of two isomeres called alpha and beta carotene. Karrer finds the structural formula to be that of a carbon chain attached to a ring at both ends. Xanthophyll, $\text{C}_{40}\text{H}_{56}\text{O}_2$, is associated with carotene and is regarded as a glycol of it. Other pigments that have a biological value are the anthocyanins (the basic substance of the red and blue flower and fruit pigments).

The foremost of plant pigments is chlorophyll. It has long been thought to be of dietary value. Its close relationship, chemically and phylogenetically, to hemoglobin has often been

referred to and as often questioned. Their chemical relationship is established by the structural similarity between chlorophyll *a* and the blood pigment hemin.

Yet another group of pigments, long known in plants but only of late attaining prominence in animals, are the *flavones*. Important also are the *flavins*. The former are yellow pigments familiar as dyestuffs; they occur as glucosides in combination with glucose. Flavone appears to be the mother substance from which come many plant pigments (such as quercetin from *Quercus tinctorius*). The flavins are yellow animal pigments of no chemical relationship to the flavones. Ellinger describes ovo-flavin and lactoflavin, the latter forming orange-red crystals.

Willstätter has shown a close relationship to exist between the flavonal pigments and the anthocyanins. The flavones are structurally related to the anthocyanins. The following formula is given for the chloride of cyanin:



The term anthocyanin was first introduced by Marquart, in 1835, to designate the blue pigment of flowers. Later, the red and purple (magenta) pigments were found to be of the same chemical constitution but existing in another form. The terminology now generally accepted is "anthocyanin" for the red, magenta, and blue glucoside pigments and "anthocyanidin" for the nonglucoside form. The glucoside in the cornflower is cyanin; and its sugar-free form, cyanidin. Agreement in terminology for the yellow animal pigments has been attained by calling the whole group "lyochromes"; and their individual specimens, "flavins."

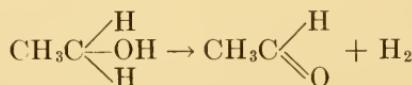
Animal pigments play a prominent role as part of the blood. Hemoglobin is the pigment responsible for the transportation of oxygen. In the oxidized form, as oxyhemoglobin, it is reduced through the liberation of oxygen; as a base, it neutralizes the carbon dioxide which the tissues give off. (There is some question as to this so-called oxidation. It appears that no true oxidation takes place but that the oxygen is, instead, simply adsorbed by the blood, *i.e.*, by the hemoglobin or the iron in it, and carried from the lungs to the tissues where it is given up, no change taking place in valence of the iron.) The two chief constituents of the blood pigment hemoglobin are hemin and globin.

The former is an iron porphyrin; its synthesis marks a great advance in research on blood constitution. The formation of hemoglobin is in a large measure dependent upon diet. A pure milk diet produces anemia (in adult animals). A trace of iron in the milk is not sufficient (hemoglobin contains iron), but a trace of copper in addition to the iron will cure the anemia. Studies on mammalian pigments, particularly in man, have been limited mostly to those of the blood, but there are a number of others of importance such as the bile pigment bilirubin and numerous pigments occurring within cells, some of which are hemin compounds. That hematin pigment which has come into prominence of late, owing primarily to the work of Keilin, is *cytochrome*. It is an intracellular respiratory enzyme, or pigment, belonging to the iron porphyrin group, as does hemoglobin. It was discovered by MacMunn (in 1886), and its existence now confirmed by Keilin. Cytochrome is said to be of almost universal distribution (in all aerobic but not in anaerobic cells).

Cytochrome proved to be more than just another pigment; it brought together the opposing views of Wieland and Warburg on respiration. The former had his hydrogen-transport theory based on a model of platinum black, wherein oxygen plays no part



(A being any oxidizable organic substance). From this point of view, the catalysis is hydrogen activation:



Warburg, too, had his model; it was of blood charcoal and capable of oxidizing various substances, especially oxalic acid and amino acids, in the presence of oxygen (see page 180 for a discussion of this in reference to adsorption and narcosis). Warburg viewed oxidation in tissues only from the point of view of oxygen activation, and he recognized but one enzyme (*the Atmungsferment*, now known to be indophenol oxidase), while Wieland considered oxidation only from the point of view of hydrogen transport but thought that various specific agencies might be responsible.

Then came the work of Keilin. He showed that cytochrome exists in reversibly oxidized and reduced forms and acts as an intermediate carrier of hydrogen. He then tried inhibitors and found that narcotics, *e.g.*, ethyl urethane added to yeast (which Warburg supposed to block the surface of the oxidizing ferment), prevent the reduction of cytochrome and do not inhibit oxidation of the pigment, while cyanide causes complete and irreversible reduction of cytochrome to such an extent as to prevent oxidation even in the presence of oxygen. Keilin also showed that the mechanisms that reduce cytochrome are mainly dehydrogenases and their substrates; the oxidizing mechanism is indo-phenol oxidase.

Thus, it seems that cytochrome is the respiratory intermediary, a role that has been attributed to numerous substances, notably glutathione when it was first isolated by Hopkins. Hopkins and Elliott, however, showed that while glutathione can function as a respiratory enzyme, it accounts for but 7 per cent of the oxygen intake of tissues. Warburg's first respiratory enzyme, an oxygen carrier, the active group of which is hemin, has now been supplemented, if not replaced, by a second oxidation ferment which he and Christian isolated (from yeast and lactic-acid bacteria); it is free from hemin bodies. Warburg is thus forced to retreat from his original position that there is only one enzyme involved in respiration. The new respiratory ferment, a yellow-red oxidation pigment, consists of a carrier of high molecular weight (50,000 to 70,000, as determined by Svedberg and Eriksson) and coloring matter which is a flavin related to ovo- and lactoflavin. It is given the formula $C_{13}H_{13}N_4O_2$.

Recently, a whole series of pigments (from yeast, liver, heart, and urine) have been described and claimed to be concerned in respiration.

Briefly summarizing, we find that the flavins (now identified as a component of vitamin B_2) are probably directly concerned in respiration, but it is the combination of phosphoflavin with a specific protein—a flavoprotein (the new yellow pigment of Warburg and Christian)—which is the important respiratory catalyst. The strongly reducing qualities of vitamin C (ascorbic acid) also make it active in respiration. The enzymes peroxidase (in plants) and catylase (in all anaerobic organisms) appear also to be significant in respiration.

The outstanding fact which particularly concerns us here is the significant role that pigments have been shown to play as enzymes (it was somewhat disturbing at the outset of these studies to call a pigment an enzyme), and to this fact can be added the equally important relationship shown to exist among the pigments, respiratory enzymes, and vitamins.

OTHER REGULATORY SUBSTANCES

Needless to say, there are many regulatory substances concerned with growth, health, and nutrition other than those mentioned in this chapter. Notable omissions are salts of the heavy metals, of which there is only a trace in tissues; and certain car-



FIG. 179.—A. Epithelium of normal adult mouse control. $\times 950$. B. Basal membrane of epithelium of SH treated skin of adult mouse. $\times 950$. (From F. Hammett.)

bohydrates, fats, and proteins. Their function is referred to in other chapters. But there is one among them that we may more properly single out for discussion here; it is the growth-promoting sulphhydryl group SH.

Sulphhydryl, SH, has come into prominence of late through the work of F. Hammett. It appears that a sulphur-containing compound, attached to proteins or protein derivatives, augments proliferative growth, but not sulphur in any form—not, for example, as the disulphide but only as the sulphhydryl. Thioglycollic acid and cysteine were used, and with them, as controls, the corresponding compounds acetic acid and alanine, containing the identical elements in the same proportion but with sulphur omitted. Sodium dithiodiglycollic acid and cystine were also tried, and in every case the number of mitoses (dividing cells) in the root tips of plants and the size of the colonies (number of

individuals) of *Paramoecium* were greater in the sulphhydryl-containing cultures. The skin of mice was rubbed with the sulphur compound. Histologic examination showed an increase in the growth of the epithelial tissue of the treated animal as compared with the control (Fig. 179). The next step was application to the human being. Here, again, the sulphur-containing compound (thioglucose) activated cell division, thus producing the healthy growth of new tissue and the healing of wounds. Thiocresol and cysteine proved to be more successful than thioglucose.

If we briefly summarize the contributions of Hammett, they are that sulphhydryl, $R-SH$, compounds stimulate growth,

while partially oxidized sulphinate, $R-S\begin{array}{c} \backslash \\ \backslash \\ O \\ OH \end{array}$, and sulphoxide,

$R-S-R$, derivatives retard growth.



Sulphur is generally regarded as a very important element in the growth of an organism (page 432). Complex substances, such as the body fluids of an embryo, may owe their remarkable growth-stimulating powers to sulphur groups. The advances in the biochemistry of growth-stimulating and regulatory substances are so great and come so rapidly that no review is adequate. The auxin work of Went is now supplemented by that of P. W. Zimmerman who finds that many substances (*e.g.*, carbon monoxide) induce the formation of adventitious roots. Murneek shows the relation between asparagine in plants and urea in animals. Harington has synthesized glutathione; and Růžička has added to the list of male hormones. Especially significant are the remarkable accomplishments in the artificial production of hormones and other regulating substances (*e.g.*, ascorbic acid and hormones of the male and *corpus luteum*).

CHAPTER XXVII

THE ORIGIN OF LIVING MATTER

The geologist tells us that life originated on earth about a thousand million years ago. The oldest known fossil is from an archeozoic pebble in a conglomerate of early Proterozoic age found in Minnesota. Apparently, no specific name has been given to this fossil. It is a blue-green alga related to the modern *Inactis* or *Microcoleus*. The fossil must be at least nine hundred million years old, if we accept, as most geologists now do, the age determinations based on the rate of disintegration of radioactive substances. Presumably, life originated at a time that, geologically speaking, was not much more remote than this.

When life began on earth, the world did not greatly differ, physically or chemically, from what it is today. The sea was less salty, and the temperature in general possibly more near that of the tropics, but in the main it was a world similar to ours of today. It is likely that certain fresh-water Protozoa and algae may still persist from the earliest periods of the earth's history, because their environment has changed so little.

Before living protoplasm could be produced, its simpler, yet still very complex, constituents had to be formed. The first significant step in the origin of living matter was the production of an organic compound from inorganic substances. We start, then, with a primeval world of water, carbon dioxide (in solution and in the atmosphere), sunlight, and mineral matter. The last mentioned will have existed both in true solution and in a finely divided, colloidal state. This is apparently sufficient to serve as the basis for the production of a simple organic substance, such as formaldehyde ($\text{CO}_2 + \text{H}_2\text{O} = \text{CH}_2\text{O} + \text{O}_2$) or possibly even a simple sugar ($6\text{CO}_2 + 6\text{H}_2\text{O} = 6\text{CH}_2\text{O} + 6\text{O}_2 = \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$) in a manner similar to that which occurs in the plant. If theories on the mechanism of food synthesis (photosynthesis) in the plant are reasonably accurate, then water, carbon dioxide, a catalyst, and solar energy are sufficient to pro-

duce organic substances from inorganic without the aid of living matter. Matter in the colloidal state is usually a very efficient catalytic agent. It will be recalled that platinum, ordinarily an inert metal, becomes a highly efficient catalyst when in the form of colloidal, or spongy, platinum. Reactions occur in the presence of such colloidal catalysts because of the tremendous surface that the finely divided material presents. On this surface, *i.e.*, at colloidal interfaces, the adsorption of water and carbon dioxide will take place and result in the synthesis of organic matter through bringing inorganic substances into molecular relations with each other. In the ancient world, the colloidal catalyst may have been volcanic dust falling into rapidly drying lakes, or it may have been the soil.

The theory that life originated on land rather than in the sea, as heretofore taught, is now rather generally accepted by geologists. The controversy is an old one, having existed among the ancients, who were divided into three schools—those who taught that life originated in fire, those who maintained that it originated in water, and those who believed that it began in soil. Fire was long regarded as alive, for it possesses such qualities of life as the need for air without which it perishes. Goethe believed in water as the seat of the origin of life, for he says:

Alles ist aus dem Wasser
 entsprungen,
Alles wird durch das Wasser
 erhalten.

FAUST.

The modern geologist, however, favors the land as the place where life began. Chamberlain first advanced the idea that life originated in the ground because the environment there favors concentration—the gathering together and building up of substances, rather than a scattering and dilution of them as in the ocean. If life actually originated on land, it must soon have migrated into shallow waters along the seashore, for it is unquestionably there that the development of invertebrates into the various higher divisions of animal life occurred. Practically all of the earliest paleozoic fossils are of marine organisms. From the sea, certain strains of living creatures then migrated to the land. Many of their offspring later sent expeditionary forces

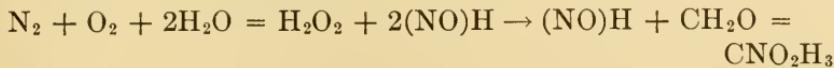
back into the sea. The best illustration of this aquatic adaptation of an animal whose ancestors were terrestrial is the reptile Ichthyosaurus. But our problem dates back earlier than the origin of living forms.

The first organic substance produced on earth may have been sugar or some substance intermediate between carbon dioxide, water, and sugar. The artificial production of sugar has long occupied the attention of chemists and biologists, not so much because of its bearing on the theoretical question of the origin of life but primarily as a solution of the secret of photosynthesis by plants and the commercial imitation of it on a profitable scale. The German chemist Emil Fischer was the first to synthesize sugar in the laboratory, but his methods were very complex. The English chemist E. C. C. Baly has gone at the problem in imitation of nature. Some years before Baly, his fellow countryman Benjamin Moore, a pioneer in the chemistry of life and an ardent advocate of the view that it was meaningless to predicate the existence of a vital force to explain phenomena of which the chemistry and physics were not understood, succeeded in synthesizing formaldehyde from carbonic acid and water, using colloidal iron oxide as the catalyst and artificial light of very short wave length as the energy. At least, he is credited with the experiment and a subsequent one in which he accomplished the same result with ordinary sunlight, using a pigment such as methyl orange to act as catalyzer of the chemical change. As formaldehyde is believed to be a stage in the process by which green plants build up sugars and starches from carbonic acid and water, Moore's experiment was apparently a definite step in artificial photosynthesis. Today, the results claimed by Moore would be questioned.

Some years later, Baly and his colleagues announced that they had succeeded in converting water and carbonic acid into formaldehyde, and the formaldehyde into sugar, using the energy of ultraviolet light. A stream of carbon dioxide was passed through water containing a fine colloidal suspension of a metal (aluminum) or a salt (nickel carbonate) and exposed to sun or artificial light. Uncertainty exists on numerous points—*e.g.*, on whether the first product formed through the action of ultraviolet light on carbonic acid, H_2CO_3 , is formaldehyde, CH_2O , which is presumably activated by light and polymerized

(united with more of its kind), yielding a carbohydrate (*i.e.*, there are two stages in the process); or the carbonic acid is converted directly into carbohydrates, formaldehyde as such not being produced (*i.e.*, there is only one stage). These problems are old ones for the botanist in his attempts to understand the chemistry of photosynthesis in the plant. There are other possible criticisms of the experiments of Baly, but whatever the actual facts may be, the underlying thought is a sound one. Only through such experimental work can we ever hope to emulate the living plant and use the sun's energy for the mass production of food.

Baly planned his experiments along classical lines, for it has always been assumed that the plant produces sugar from carbon dioxide and water, with formaldehyde as a possible intermediate product. Baudisch believes that food manufacture in the plant is more complicated and may involve the immediate introduction of nitrogen without sugar being first formed. He also introduces atmospheric nitrogen into the reaction. Botanists have long considered this possibility but have always rejected it, being of the opinion that the limitless supply of nitrogen in the air is not made use of directly by the plant. Baudisch regards atmospheric nitrogen as combining with atmospheric oxygen and water to form nitrosyl which unites with formaldehyde, producing formhydroxamic acid, thus:



It may be that the synthesis of protein in the plant and the synthesis that eons ago led to the formation of living matter do not involve the production of sugar; a nitrogen-containing compound, a protein, or a protein derivative may instead have been produced directly from water and carbon dioxide.

We started our story of the possible origin of living matter on earth with a world in which water and carbon dioxide were already present. E. E. Free takes us back a little earlier and outlines a possible course of the synthesis of the first substance formed. He says that when the earth grew colder, the first chemical compound produced was probably the oxide of titanium, but this is of no known importance to the story of life. The next two compounds were carbon monoxide gas, CO, and either

prussic acid (hydrocyanic acid, HCN) or the closely related and interconvertible compound that chemists call cyanogen, CN. These are the compounds that form when a hot mixture of the three gaseous elements oxygen, hydrogen, and nitrogen is allowed to cool slowly. At a somewhat later stage of the cooling, another chemical reaction occurred. Hydrogen gas and oxygen gas combined to form water, or perhaps at first steam, while the earth was too hot for any liquid water to exist, which would later condense to water. Prussic acid gas is soluble in water. Accordingly, the primitive ocean must have absorbed considerable amounts of this material. The water would also dissolve a small amount of the carbon monoxide gas. In this ocean of dilute prussic acid overlaid by an atmosphere containing large amounts of carbon monoxide gas, the first constituent of living matter possibly arose. Protoplasm contains large amounts of proteins. These, and therefore protoplasm itself, can be broken down into the simpler nitrogenous constituents, the amino acids. The amino acids, of which the simplest is glycocoll, contain carbon, hydrogen, oxygen, and nitrogen. It is certainly more than a coincidence that this compound, glycocoll, may be produced in the laboratory by a succession of chemical reactions among the three substances which we have seen were present in the primeval ocean—prussic acid, carbon monoxide, and water. During the millions of years that were to elapse before the period when we find actual traces of life in the rocks, there was ample time for simple nitrogenous substances such as glycocoll to undergo changes and combinations—to be built up into more complicated forms and ultimately into substances similar to protoplasm. While the individual steps cannot be traced, it is possible that by some such path as the one outlined by Free did organic matter first arise on earth.

While carbohydrate or protein may have been formed under high temperatures, it is probably not so likely a method as the one followed by the plant. There is also the possibility that the path along which living matter developed may have been other than any of the hypotheses so far given; for example, sugar may have been formed by the combination of free carbon and water instead of by the reduction of carbon dioxide in water; or, again, if a hydrocarbon was the first organic substance formed, it may have arisen through the combination of free carbon and hydro-

gen, and sugar have come later. We do not know, but any of the paths so far suggested is possible.

In seeking a correlation between living and nonliving matter, we are sure first of all of the intimate association of life and organic substances. Protoplasm contains, on the basis of its dry weight, 95 per cent of organic matter (amino acids, purins, sugars, albumins, nucleoproteids, nucleic acids, globulin, lipoprotein, fat, phytosterin, and phosphatids) and 5 per cent of inorganic material. It seems certain also that the synthesis of protoplasm and subsequent reactions within it occur in a colloidal medium. As Findlay says, we must recognize the essential importance of colloidal matter in connection with the phenomena of life. Matter in the colloidal state is the vehicle of life.

A way in which living matter might come from nonliving having been tentatively accepted, there next arise the interesting questions Did this event occur but once, or is it now taking place? Is our earth the only place on which it has occurred? Can it be produced at will in the laboratory? And wherein lies the distinction between the living and the nonliving? These questions will be considered in order, with the realization, however, that none is definitely answerable, though several permit consideration on strictly scientific grounds.

An offhand answer to the first question would be that, once formed, there is no reason why living matter should not again be produced; indeed, the process may now be going on. Reflection leads to another answer. Certain special conditions were necessary for the production of a very complex substance. The conditions existing in the experiment of Baly still exist on earth; but if living matter arose in the manner postulated by Free, *i.e.*, at a time when there was an ocean of dilute prussic acid and an atmosphere of carbon monoxide, then it is hardly likely that life, as we know it, will ever be produced again on this earth.

The question whether there is life on other planets has often been asked. Again we do not know; but as other worlds are made of the same material as is ours—meteors testify to this—there is no reason why the conditions that produced life on our globe, no matter how special, should not have occurred on other worlds. Planets are particularly suited for life such as that on our earth. The solar system of which we are a part possesses eight planets; one of these supports life and another—Venus—

may be habitable. The formation of planets, in particular life-supporting planets, may be the usual course of events for other stars, just as it has been for our star—the sun. Possibly a planet on which life can exist is a very rare occurrence, but the astronomer J. G. Porter says, with a touch of irony, “Surely the success of the noble experiment of life on the earth has not been so notable that we may not hope for better results elsewhere.”

The question whether or not living protoplasm can be artificially produced is one upon which many biologists have speculated and usually energetically answered in the negative. We must grant the possibility of doing it, with sufficient knowledge, but he who claims that protoplasm can be made in the laboratory, now or at some later time, might better ask a child to construct a chronometer.

Our last question is Wherein lies the distinction between the living and the nonliving? This cannot be answered with any more success than have been the other questions. It is not usually difficult to point out that which is living and that which is nonliving, but to say wherein the difference lies is quite another matter. Reactions that we are in the habit of associating with life are, on analysis, usually found to be typical of nonliving systems as well. Locomotion, respiration, growth, irritability, self-repair, and, in a broad and crude way, even reproduction and memory can be duplicated in nonliving systems. Thus, charcoal respites in so far as it can combine oxygen with carbon and form carbon dioxide. Indeed, the similarity is even greater—when oxalic acid is shaken with blood charcoal, the acid is oxidized. The charcoal acts catalytically, and not only this; it may be anesthetized—poisoned—by cyanide and inhibited by methane just as are living cells (page 179). The analogy is not merely a superficial one; there is some common factor involved.

Other processes that appear to be characteristic of living matter are also common to nonliving matter. Crystals grow. The self-repair of a “wound” is accomplished by the Traube precipitation membrane (page 185). A kind of “memory” exists in jellies (see page 145) where a previous event determines present action. Such analogies are often inexact, but equally often they give us precise counterparts and in any case bring out, what is

certainly true, that many so-called vital events take place in nonliving systems.

We can distinguish living matter from nonliving only by selecting a number of properties all of which living matter possesses, but only one or two of which are possessed at a time by any one nonliving system. Four such properties are absence of equilibrium (living matter is never in a state of equilibrium, for equilibrium means death, while all nonliving systems ultimately attain such a state); adjustment (living matter is always adjusting itself to its environment, while nonliving matter rarely does so, though it may respond to certain environmental factors such as gravity); heterogeneity (this is attained to a high degree by living matter and never so much so by nonliving matter); and the maintenance of a definite form (this is sometimes disturbed in living matter and, on the other hand, regularly and perfectly attained by nonliving matter, *e.g.*, crystals; but as a whole it is more characteristic of life).

Having failed to find any one property that distinguishes the living from the nonliving, we may now inquire if there is any one material substance typical of life. Early biologists, influenced by the vitalism of their time, recognized special vital bodies in protoplasm which give to it the properties of life. The French naturalist Buffon conceived of gigantic living molecules termed "biogens." Haeckel, Darwin, and others postulated similar units, as has been noted (page 9). The distinction between living and nonliving matter would, on the basis of these older hypotheses, lie in the possession of special vital bodies by the living substance. But such speculative ideas are no longer seriously considered, though they are not far removed from some modern thoughts. Numerous physiologists have expressed the belief that a definite substance or group of substances represents the ultimate living material. Leathes states that proteins are generally considered the most important components of protoplasm, and Pauli lays emphasis on them as substances that possess many of the characteristics of living matter. Thus, the opinion prevails that the ultimate living substance is a protein complex. The constituents of this complex may be of the nature of enzymes. Many have commented on the striking similarity between enzymes and living matter. The French microbiologist Duclaux stated that the bacterial cell

carries on its activities entirely through its enzymes, the "cell itself" being relatively inert. Perhaps the cell itself *is* the collection of enzymes. Enzymes are catalysts. Realizing the remarkable properties of catalysts, the Swedish chemist Berzelius advanced the idea that life is the resultant of the play of catalysts. In his day, organic catalysts were as great a mystery as life itself. The extraordinary similarity between enzymes and living matter is seen in a comparison of the reactions of the two. Both are destroyed by heat, light, and chemicals. Heat is destructive in each case at the same temperature (50°C.). No chemical is known that is injurious to bacteria and yet without action on any enzymes. When dry, both enzymes and bacteria are highly resistant to heat, and both are resistant to cold to an astonishing degree.

Opposed to the school that postulates a highly complex substance peculiar to living matter is that school which regards all the constituents of protoplasm as lifeless when considered individually; only in the associated coordinated state does life result (see page 10).

While this may be true, it is still possible to distinguish between strictly nutritive matter in the cell (*e.g.*, fat droplets) and the active kinetic material. Living protoplasm has its fuel and a mechanism for converting the fuel into energy. While there is no settling the matter, and while we may be forced to grant that the water in protoplasm is alive because it is part of a living system, that is to say, as much alive as any other part of the system, yet it does appear to be true that living matter contains a great number and variety of protein-like substances which seem to be peculiar to it. Thus, Hammarsten wrote that tissues and cells consist mainly, *i.e.*, fundamentally, not of proteins of our common experience, such as globulins and albumins, but of highly complicated ones, particularly those containing sulphur, phosphorus, and iron. Alone, these proteins are no more active than is a locomotive without its coal and water. Living matter is unquestionably a system. The probable possession of certain proteinaceous substances peculiar to itself and the great complexity of the system as a whole are the only material differences between living and nonliving matter.

If there is no *one ultimate* living substance in protoplasm, and living matter is simply a very intricate and highly organized

mixture of nonliving matter, then it would be interesting to know if there is a gradual gradation in complexity from the living to the nonliving, that is to say, if it is true that degree of complexity is the only distinction between the living and the nonliving, just when is this degree reached, and are there existent bodies which are intermediate in complexity and therefore belong to neither the one nor the other state? Findlay expressed himself in opposition to this viewpoint when he said that there is no continuity between inanimate and living matter—rather is there a distinct and sharp break in the curve of relations. Life is a new factor—a new set of potentialities—introduced into inanimate matter. But there is another possibility. Recent work on those remarkable substances known as filterable viruses suggests that they perhaps constitute a link between the living and the nonliving. Bacteriophage is a substance rather like the viruses. Some have said that it is "probably an organism," "self-perpetuating," arising independently in bacterial cultures which it then destroys; others view bacteriophage as a product of the bacterial culture. Then there are the filterable forms of bacteria, which, though filterable, are to be clearly distinguished from the viruses, for the viruses will not grow or multiply on a lifeless medium. The filterable viruses are also intermediate in size between living bacteria and nonliving matter; they come between the largest protein molecules and the smallest living bodies (coccus bacilli).

Filterable viruses are colloidal, for their aqueous suspensions are turbid (show the Tyndall cone), but their particles are below the limit of ultramicroscopic (dark-field) observation, and they pass through very fine filters. The size of the particle has been estimated to be $25 \text{ m}\mu$, which is about one-tenth the size of the smallest bacillus. Such a particle would consist of 200 to 400 protein molecules of average size, which is but five to ten times the diameter of a maximum-sized molecule. The hemoglobin molecule is now estimated to be about $5.5 \text{ m}\mu$ in diameter. Whether or not viruses are alive is still a debated question, usually answered in the affirmative. A diameter of $25 \text{ m}\mu$ or a content of some 200 molecules does not leave space or molecules enough for any but relatively simple chemical reactions. But the chemical reactions in the smallest bacterium are probably also less great than in a single tissue cell.

If we group all substances that are on or near the border line between the living and the nonliving, we obtain a series similar to the one given by A. E. Boycott; there are first the simple proteins, then the nonliving enzymes which cannot multiply, then "enzymes" (like lysozyme) which can multiply in the presence of bacteria, then nonpathogenic viruses of the type of bacteriophage, pathogenic viruses, filterable bacteria, and finally visible bacteria. In such a series, it is impossible to say where life begins and where it ends.

Vitalism and Mechanism.—The constituents of protoplasm, which are the material basis of life, may serve merely as the medium in which certain vital forces play. The possible existence of a vital force apart from those earthly ones that control all other natural phenomena has long been entertained and has given rise to the philosophy known as *vitalism* (or *historism*) in contrast to *mechanism*. Early religious tendencies led scientists to the belief that plants and animals differed from nonliving matter in that they were controlled or operated by a *spiritus vitae*, or, as the Germans call it, a *Lebenskraft*. Such a *vital force* is the basis of the philosophy known as vitalism.

There are two points of view in regard to vital force; one (*e.g.*, that of Driesch) holds it to be distinctly not of this world (entelechy is "neither a kind of energy nor dependent on any chemical material"), and the other (*e.g.*, that of Rignano) holds it to be of this world and therefore to be classed with other forms of energy only different from them—of the same family but a new species, so to speak ("life is a form of energy *suis generis*"). The former point of view has the virtue of being definite and beyond reach, but the latter, in attempting to harmonize vital force with known forms of energy, falls into an error. All energy is additive; chemical energy can be added to electrical, and electrical to radiant, etc.; that is to say, the total energy of a system is the sum of the separate energies ($E = \Sigma E_i$). As vital energy cannot be added to other forms, it is consequently not energy as understood in physics.

A vital force was presumed to give to plants and animals the power to synthesize organic substances which could not, so it was supposed, be artificially produced in the laboratory. Organic chemistry was so called because it was thought that the carbon compounds with which this branch of science deals were made

only by living organisms. The first step away from this vitalistic concept of the origin of organic matter was the synthesis of urea by Wöhler in 1828. The experiment was recognized, but for another quarter of a century it was held that while the superior intelligence of man made it possible for him to synthesize organic matter, such synthesis was possible in nature only because of the vital force which living things possess. Shortly after the middle of the last century, Berthelot wrote, "The objective of our science is to banish 'Life' from the theories of organic chemistry." Thus was inaugurated the revolt against vitalism. Brought on by the successes in organic synthesis, it went so far as to proclaim the possibility of the artificial synthesis of all compounds. Many of the younger biologists fell into line with the mechanists among chemists and boasted of the production of living matter in the near future. J. Loeb expressed the opinion that "something like living matter" would be compounded in the laboratory within a very few years. Perhaps the expression "something like" left a loophole out of which to escape in case the substance produced did not fulfill all the requirements of living matter. Even more confident have been some recent utterances to the effect that having "gone a long way toward understanding the composition of an amoeba [the statement could be challenged], it will not be fifty years before we can build a single-cell organism like the amoeba." Such predictions express a hopefulness that experience does not support. Life is, to say the least, a new departure and a very extraordinary one. On the other hand, when its simpler happenings lend themselves to scientific analysis, they prove to be as subservient to physical and chemical laws as are nonliving systems.

When challenged to accept one of two concepts neither of which satisfies fully, we seek a third interpretation.

The experimental scientist is often loath to turn to the philosopher for a suggestion on the solution of his problems, but this would appear to be the best way out of the present situation. One can, of course, accept the vitalistic concept unequivocally and agree with those who state that the modern physics has done away with mechanism; or one can recognize mechanism and agree with the mechanists of the last century who thought that their modern physics had done away with vitalism. Let us rather not

deny mechanism or vitalism but ask the philosopher tentatively to interpret life for us. Philosophically inclined biologists see in *emergent evolution* an escape—though it is but an escape—from the mechanistic-vitalistic dilemma. The name (not a fortunate one, as evolution has long been accepted among biologists, and all evolution is emergent) designates a point of view expressed by C. L. Morgan. Some years previously, G. H. Parker advanced a similar idea under the name of "organic determinism," or "organicism," in which life is viewed as the outcome of a specific organization of substances, each commonplace in itself; in other words, when organic molecules are assembled in a certain way, they exhibit properties quite unlike those which they show when they are brought together in another way. For example, glucose is a sugar of such and such qualities not only because it is made up of carbon, hydrogen, and oxygen but because of the way in which these elements are put together. Many other compounds consist of the same elements, yet they are not glucose. No matter how much knowledge we may have of carbon, hydrogen, and oxygen or of other compounds consisting of these elements, it will avail us nothing in an understanding of glucose. In brief, a whole is more than the sum of its parts, not merely because of complexity but because in the functional whole we have another type of system.

The American philosopher E. A. Singer would probably say that we have no need for new names because the idea that they here express is old, in one form or another, and may better be stated as follows: Living matter is a mechanism obeying all natural laws, but it is incapable of definition or explanation; that is to say, everything that goes to make up a living system is mechanical (materialistic), and the individual parts of it are definable, but the collective all is indefinable. (Materialism is a somewhat better term than mechanism, for the latter implies that living matter is a machine like a watch, while materialism states only that it is wholly physical and chemical in nature, without denying that it may be mechanistic.) Such a viewpoint recognizes the possibility of producing protoplasm artificially in the laboratory but denies the probability.

Democritus stated that there is nothing in a natural system that does not or could not exist in a mechanical one. Aristotle denied this by stating that there is something of a nonmechan-

ical character in a living system. Kant and Singer accept both of these viewpoints yet recognize that they are of two distinct kinds of classes (classes of classes), or universes of discourse, which cross at one point where, therefore, they are not incompatible.

We might view the matter in this light: A sundial, an hour-glass, and a clock are quite different types of mechanical systems, yet all have one function; at this point they meet. As mechanisms they are quite distinct; as timepieces, they are members of the same functional class. The point at which they meet is nonmechanistic, for a function is not mechanical. Thus, life as a physical and chemical system is mechanical. Its individual parts we can understand. As a living functional system, it is nonmechanical. Its whole we cannot understand.

The whole as an entity and its function as a property depend upon an arrangement of parts which is organization. To this fundamental property of living (and nonliving) matter we return again and again. The mechanism is what it is because of the way in which its parts are put together. Organization is characteristic of all functional systems, and as such it is as much an entity as are matter and energy.

In our enthusiasm for a generally acceptable philosophy of the physical basis of life, we must not exaggerate the property of organization by elevating it to a position far above that of other properties. In a sense, organization is above them all, but it is still within this world. Among the ablest of biologists of vitalistic belief is the English physiologist J. S. Haldane. He shows very well how the concept of organization or coordinated activity is so essential a part of every living thing. In criticizing a treatise wherein blood is regarded as a physicochemical system (which it certainly is), he called attention, as did Claude Bernard, to the important fact that the blood of a living animal is kept remarkably constant in its properties by the influence of the organs of the body upon it, it, in turn, maintaining coordination between the organs. He adds that to disregard the principle of coordination is to disregard all that is characteristic of life and that a study of blood apart from the body is not a study in physiology but only a study in physical chemistry. Now, Haldane is right when he says that to ignore coordination is to ignore one of the most fundamental principles of life; but

the mechanist—the physicochemical biologist—does not do this, if he is a good experimenter, any more than a good clockmaker ignores the coordination of all the parts that make up a clock. In stating that a whole is greater than the sum of its parts, we do not thereby endow the system with a property apart from this world.

A philosophy wherein living matter is interpreted as an organized functional system is not only an escape from a too rigid acceptance of either the mechanistic or vitalistic concepts, but it is also an escape from the fatalistic attitude of those who bemoan the harsh severity of experimental science. There has been of late a very evident drift away from the philosophy of Helmholtz, who declared that "the final aim of all natural science is to resolve itself into mechanics."

The reaction to mechanism set in early—if indeed it has not always existed—thus, William Keith Brooks objected to the thoughts of his mind being regarded simply as the "rattle of machinery." Kepner has more recently stated that the one-celled animal Amoeba meets contingencies (see page 58): "This fact carries us beyond science, whether we like it or not." Jennings says, "Emergent evolution does away with that monstrous absurdity that has so long been a reproach to biological science—the doctrine that ideas, ideals, purposes have no effect on behavior." The pragmatism of James—which sees in evolution the development of intellect, reason, emotion, and will, purely from the viewpoint of a relation to nervous systems, sense organs, and general complexity of structure—has been severely criticized on the ground that it will lead to the debasing concept that ideals, and truth itself, are merely an adaptation to a useful purpose. Pragmatism claims that everything must find its place in a general scheme of usefulness. In a sense, this is all true and perhaps to be desired, but that the experimental method, a mechanistic interpretation of vital processes, and the philosophy of functional organization lead to a system of ethics "more characteristic of a savage than a civilized state" is nonsensical. If science has failed to make its additions to the betterment of mankind in the same way as have the humanities, then it is not the experimental or mechanistic method that is responsible, nor, indeed, is it science in any form, but rather man's application of the truths unearthed through science.

A severe mechanist may yet be more humanistic in his attitude than the most ardent vitalist. The difficulty is that we have learned to control nature before we have learned to control ourselves.

Certain phenomena, such as gravitation and life, have so far defied all attempts at a purely mechanical interpretation. Perhaps they will continue to do so. - We need never hope for a machine that will reproduce the thoughts of Newton, the emotions of Beethoven, or the inspiration of Michelangelo. Yet our only safe method of procedure in science is the experimental and mechanistic one.

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